LABORATORY EXERCISES

FOR

INTRODUCTORY BIOLOGY

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of Education

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FOREWORD

THIS MANUAL was prepared by Mr. L. R. Tolman with the assistance of Dr. B. Hocking and the other members of the Subcommittee on Biology of the Curriculum Committee of the Alberta Department of Education. The membership of the Biology Subcommittee is as follows:

Mr. O. Massing, chairman
Mr. Robert E. Ayling
Mr. Cy Hampson
Dr. B. Hocking
Mr. J. S. Hrabi
Mrs. M. Jones
Mr. E. Meyer
Mr. I. P. Stonehocker
Mr. L. R. Tolman

Much of the content represents a revision of the material in the LABORATORY MANUAL TO ACCOMPANY BIOLOGY 20, Department of Education, 1962. The permission of the American Institute of Biological Sciences and of Dr. J. E. Moore and Dr. V. Lewin of the Department of Zoology, University of Alberta in Edmonton to use keys from their work is gratefully acknowledged.

POISON WARNING

Many substances used in the biological laboratory are poisonous. This applies especially, of course, to materials used to kill and preserve animals. Some materials may be infective.

When in doubt about a substance treat it as if it were poisonous. Always wash your hands thoroughly after working in the laboratory and before eating or drinking. Attend carefully to any instructions or precautions which may be given on a container.

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NOTICE TO THE TEACHER

Certain of the laboratory exercises in this manual require attention beforehand in order that materials may be obtained and ready for class use at the desired time. Many of these materials should come from the field. Field work is an essential part of this course and a field trip early in the school year should serve this additional purpose. Information on field work appears in Appendix A.

Certain titles in the Table of Contents have been marked. The marks indicate:

* *requires materials to be collected in the field at an appropriate season*

** *requires special preparation in advance of the laboratory period. Where necessary, instructions for the special preparations are provided in the appendix of this manual.*

PREFACE

This laboratory work book has been designed to accompany the text *Modern Biology,* by Moon, Otto and Towle, published by Holt, Rinehart and Winston, 1963.

As a beginning student in biology you no doubt look forward to exploring the many mysteries of living things. This is good. Without curiosity and enthusiasm our lives would be dull indeed. However, idle curiosity takes one nowhere. It is not enough to be curious—one must do something to pursue knowledge and understand why things are as they are.

One does not become a biologist simply by taking one or more courses in biology. Nor is the goal achieved merely by routine performance of laboratory exercises. It is necessary to develop safe and appropriate techniques. It is necessary to know what you are doing and why. Above all, it is essential that you give of yourself—ambition, understanding, and integrity are the ingredients of rewarding laboratory work.

There are 37 exercises in this workbook and most of them can be completed in a forty-five minute period. Take pride in your work. Prepare for each laboratory period in advance. Problems which you encounter must not be set aside. Try to find answers by further study and experimentation. Finally, do not neglect to raise questions in class. Answers often are brought out through discussion under the guidance of your teacher.

INTRODUCTION

The Exercises—The laboratory exercises in this workbook are of three types:

1. Exercises to acquaint the student with a specific piece of apparatus, a particular laboratory technique, or some basic observation.
2. Exercises to develop special basic skills. These are exemplified by the dissection exercises. Of course one does not dissect an organism just to develop proficiency in dissection but also to study and become more intimately acquainted with the organism.
3. Experimental exercises. These are intended to encourage investigation in various fields. Types 1 and 2 are rather prescriptive; in this third group students are given scope to attack problems and thus gain insight into scientific methods.

Material—Whenever possible use living or fresh frozen material. This is not only much easier to work with but, even after you have killed or anaesthetized it, is much closer to the live plant or animal—which is what biology is about—than is old preserved material.

Try to develop a feeling for the needs of living plants and animals and to maintain as many of these as possible in the laboratory. Some culture methods appear in an appendix to this manual. Your biology laboratory should be alive with plants and animals. Every member of the class can help to make it so. Try always to relate what you are doing to the living organism.

Dissection—To dissect means to cut apart (Latin *dis-,* apart; *secare,* to cut); note that the "s" is double and the "i" short. If you pronounce it with a long "i" you may spell it wrongly and in any case this would mean to cut twice or to cut in two, which are both things to avoid in good dissection.

Dissection has always been the most important technique in the study of the structure of plants and animals. It requires care, which everybody can exercise, some foreknowledge, which this manual and your text and teacher will give you, and skill which will come with practice. There is no short cut to good dissection, but these suggestions may be helpful.

Always explore before you cut so that you know where your cut will lead you. It may be necessary too, to make rough preliminary drawings at various stages before cutting. Keep all instruments clean and sharp. Use the appropriate instrument for each step; neither a meat axe for an insect nervous system nor an eye scalpel for the guts of an elephant. Never use a scalpel to cut unsupported soft tissue; if support cannot be arranged, use scissors. Use pins liberally to hold loose parts and tissues out of the way of further operations. Wash off frequently but carefully.

Drawings—Drawing is a very important procedure in the study of the structure of plants and animals. This is scientific drawing, and it attempts to represent in two dimensions on paper what is usually (except for sections on slides) a three dimensional object. It is not artistic drawing and calls for no imagination or other artistic ability; in fact imagination must be strictly avoided. Do not, therefore, be discouraged if your first results disappoint you—they should. Keep trying until you are satisfied that what you have on paper truly represents what you saw.

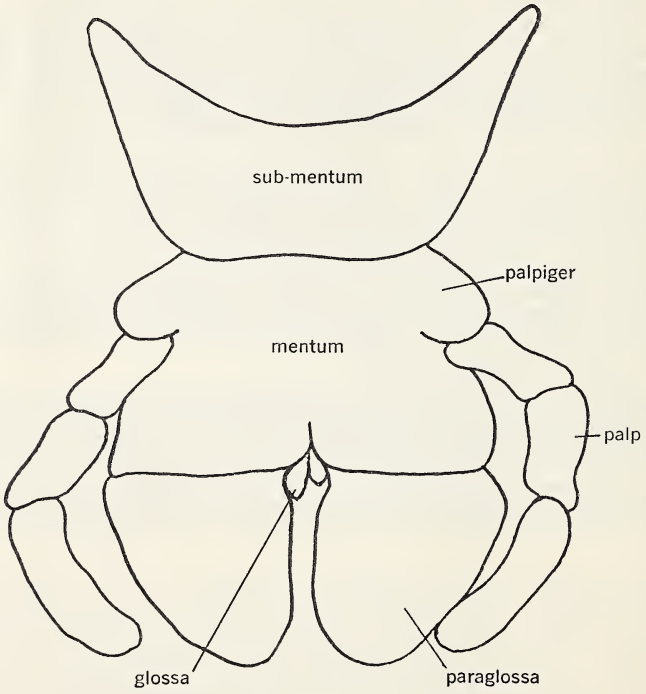
It is by this translation from three dimensions to two that you learn the structure of the plant or animal. It follows that much of the value of drawing is lost if you copy somebody else's drawings or those on charts or in books. Furthermore mistakes are perpetuated and multiplied. It was in this manner, through failure to draw from the original, that drawings of a honey-bee by the ancients became converted to a lily, the fleur-de-lys, in the Middle Ages.

Decide on the scale and aspect you are going to use before starting and indicate these beside the drawing. Make the scale as large as possible and show this either as a factor of linear magnification or reduction as x 5 or x 0.5, or as a line with the actual size of the structure or organism marked off on it. When drawing dissected parts, show their relationships to the whole organism.

Do not be in a hurry. Examine the specimen thoroughly before you start drawings and refer back to it constantly as you progress. Use a firm or hard pencil (F, H, or 2H) and keep it sharp. If you find it necessary to feel for a line sketchily at first, put it in firmly when you have made your decision and erase the other lines.

Label drawings as fully as possible and as clearly as possible. Where the name is short and the part large it may often be written on the part. Otherwise use ruled guide lines, preferably much lighter than those of the drawing, take them out of the drawing by the shortest route and never let them cross. Print all names in lower case letters across the page so that they can be read without turning.

Shading, stippling, and coloring call for special skill. They may obscure lines and permit errors of lines to be overlooked; do not attempt them at this stage in your biological studies.



INSECT MOUTHPARTS

Labium of Grasshopper
Melanoplus sp.
Ventral aspect x20

Fig. 1

INSECTA
ARTHROPODA

THE MICROSCOPE

Object—to learn the parts of a microscope and their functions.

Materials—compound monocular microscope.

The microscope is an instrument designed to make it possible to see and examine objects too small to be seen or examined with the naked eye. The type in most common use is the compound monocular microscope. With this type a two dimensional image, reversed as in a mirror, is seen with one eye. Usually the object is translucent and the light passes through this before reaching the eye.

Another type, the stereoscopic binocular microscope, is more satisfactory for manipulative work. This type is essentially two microscopes, one for each eye. The image is three-dimensional, is not reversed, and is viewed with both eyes. Usually the object is opaque and is seen by reflected light. The binocular microscope provides lower magnifications to inspect objects in a wider field of view.

Procedure—(a) It is important to know when using a microscope how much the object is magnified. To get the total magnification, multiply that of the objective by that of the eyepiece or ocular. Check the objectives and ocular on your microscope and calculate what magnifications are possible. Record your findings.

Equally important in a microscope is its definition, which, together with magnification, determines resolving power. This is the power to separate the images of adjacent objects. On the road after dark, when you first see the lights of an approaching car, you see only a single point of light; after a while the lights are near enough so that you can see them separately. At this distance your eyes are able to "resolve" the two lights. Two points less than 0.1 mm apart cannot usually be resolved by the unaided eye, but can readily be separated under the microscope. High quality optical microscopes enable one to see objects down to about 0.1 micron in diameter. (Greek letter mu=micron), (one mu=0.001 mm.)

One other factor is important in the use of the microscope: the realization that the working distance, the distance between the object viewed and the bottom of the objective, becomes smaller as the magnification is increased. Skill in the use of microscopes will prove valuable to you in many of the observations and experiments you will do in biology.

(b) The parts of the microscope: be sure that you know the following parts of the microscope: base, arm, stage, tube, nosepiece, eyepiece or ocular, high and low power objectives, diaphragm, mirror, fine and coarse adjustment focusing knobs. Examine the microscope with which you have been provided, locate and examine each of the structures named. Note that many modern microscopes have stage focusing, that is, the focusing knobs move the stage instead of the tube. This means that they have to be turned in the opposite direction to reduce the working distance. Decide which type of focusing your microscope has and familiarize yourself with its operation.

(c) Setting up a microscope: carry a microscope with two hands. Hold the arm with one hand and place the other hand under the base. Set it down gently, the arm towards you, the stage away from you. Put the low power objective, the shortest one, in line with the tube; it will click into position. When you have finished using your microscope always turn the nosepiece so that the low power objective is in line with the tube. You will notice that when you turn the nosepiece so that different power objectives are in line with the tube, each setting is aligned when it clicks into position. Move the mirror so that it reflects light from a lamp or a window (preferably a north window) upward through the opening in the stage. Do not use direct

sunlight; it will be too bright. Look through the microscope and adjust the diaphragm so that the round field of view is evenly illuminated without glare. It is better to keep the lens surfaces of a microscope clean by handling it carefully than to clean them often. Do not touch the glass surfaces with your fingers or turn the objectives down into liquids. Use only a clean camel hair brush followed by lens tissue to clean the glass surfaces.

Make a neat sketch of a side view of the microscope with which you have been provided. Label all of the parts listed in part (b) above.

LAB. 2

THE USE OF THE MICROSCOPE

Object—to learn how to use a microscope.

Materials—microscope, slide—1, cover slip—1, medicine dropper—1, lens tissue or clean cotton cloth, shaded newspaper picture, paragraph of newspaper printing, 6″ plastic rule—1.

Procedure—(a) Clean a glass slide with water, holding it by the edges, wipe clean and dry. Cover slips are fragile; **handle with care!** Clean the slip by gently rubbing it between a clean cloth held between the thumb and forefinger. Cut or tear from a newspaper a piece of shaded picture. The piece should be about ½-inch square and include different degrees of shading (light to dark). Observe the shading with the naked eye and then place the piece of paper on your slide. Put one or two drops of water from an eye dropper on top of it. Let this soak in, then cover it with a cover slip. Some skill is required to do this without trapping air bubbles. The best method is to hold the cover slip at about 45 degrees to the slide, bring one edge down first and then use a pencil point under the upper edge to lower it slowly over the specimen. A very gentle tap on the cover slip with the pencil will dislodge air bubbles at the edge.

(b) Put the wet mount on the stage of the microscope so that the paper is in the centre of the opening in the stage. While looking at the microscope from one side, use the coarse adjustment to lower the tube or raise the stage until the low power objective is a few millimeters above the cover slip. While looking through the eyepiece, raise the tube or lower the stage until the image comes into view. With the fine adjustment make the focus as sharp as possible. If you are right-handed you will probably find it best to look through the microscope with the left eye. Keep the right eye open; you will then be able later to learn to draw without taking your eye from the microscope.

Describe and compare the appearance of the shading as seen with the naked eye and as seen under low power. Remove and clean both slide and slip.

(c) Prepare another wet mount of a small letter "e" torn or cut from the piece of newspaper. Mount your slide with the letter "e" right side up. Focus your microscope using low power so that you can see the image clearly.

(1) Which way round is the letter "e"?

(2) Move the slide on the stage from right to left. Which way does the image move?

(3) Move the slide away from you. Which way does the image move?

Note what occupies the centre of the field of view. Revolve the nosepiece so that the high power objective clicks into place. If the focus remains fairly sharp, the microscope is said to be parfocal, but some further adjustment of both focus and light may be necessary for the best possible image.

If necessary readjust the mirror and diaphragm to restore an even illumination without glare. If the slide starts to dry out add a little water. Do not raise the cover glass but put a drop of water from an eye dropper at the edge of the cover slip; it will be drawn under by capillary action.

(1) Do you find the same part of the image in the centre of the field of view?

(2) Compare the sizes of the fields under low and high magnification.

(3) Compare the relative illumination of the fields under low and high magnification.

(d) Place a plastic rule on the stage and hold it with the stage clips in such a way that the edge with the smallest graduations (mm. or inches) may be observed under low power. Focus the microscope so that you can clearly observe these graduations and thereby measure the size of the field.

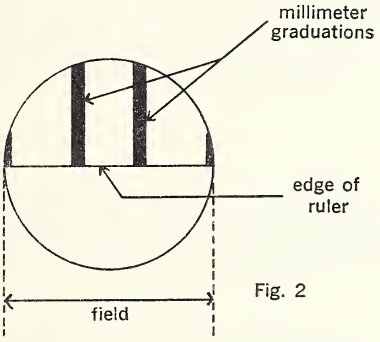


Fig. 2

Fig. 2 shows what you may see. You will notice that three spaces show in the field. If the total magnification used is 20x this would mean that the diameter of the image of the field would be 3 x 20 or 60mm. or slightly over 2¼ inches. Thus if one were examining an organism which extended about half way across the field at 20 magnifications, the drawing of the organism should be about 1⅛" long if it is drawn to proper scale. Of course, if one wanted to draw a larger diagram, he could make it perhaps four times (4½" long) and mark the magnification at 80x. This is a very important procedure in microscopy. You will have many occasions to use this information in succeeding laboratory exercises.

Using this technique, measure the size of the field of your microscope under low power. Record this information in a place where it will be available for quick reference.

(e) A microscope is an expensive instrument and needs careful handling. Do not stand it too close to the edge of a table or bench. At the end of a laboratory period, wipe the microscope stage should it be wet. Turn the low power objective into a position about 1 centimeter above the stage. Check that the slide clips do not extend beyond the sides of the stage and return the instrument to its proper storage place. Clean all slides and slips which you have used.

LAB. 3

MICROSCOPIC ORGANISMS

Object—to observe various micro-organisms found in nature and learn about their structure.

Materials—microscope, slide—1, cover slip—1, pond water cultures (see Appendix A) or hay infusion, methyl cellulose solution.

Procedure—Put a drop of water from the scum formed on the surface of the culture described in Appendix A. This water contains large numbers of microscopic organisms. Place a cover slip on the drop of water. Place the slide on the stage of the microscope with the drop of water centered over the opening. Adjust the mirror and

diaphragm so that you can see a clear, even field without glare. While looking through the eyepiece, use the coarse adjustment to bring the micro-organisms into view. Use the fine adjustment to make the image as sharp as possible.

Adjust the sub-stage diaphragm in order to vary the amount of light passing through the optical system and thus bring out maximum detail of the organism viewed.

(1) What setting of the sub-stage diaphragm permitted you to see maximum detail in the organism examined?

Locate one of the larger organisms in the field and try to follow its movements through the water. This requires some skill; do not be discouraged if you do not succeed at the first trial.

(2) In which direction must you move the slide, as compared to the direction in which the animal appears to move?

In order to follow the movements of the microscopic animals in the field, it is necessary to use both hands—one on the slide and one on the fine adjustment.

(3) As the animals move about, they not only change their position in the field, but they also move in and out of sharp focus. Why?

(4) How does the apparent speed of movement of the animals vary with the magnification? Why?

The movement of protozoa and other microscopic animals can be slowed down by viscous solutions. Put one drop of methyl cellulose solution and one drop of micro-organism culture together on a slide. Mix both drops together, and add a cover slip. Examine the slide and notice the slowing effect produced by this procedure.

Project Suggestion—You may now wish to examine slides prepared from the other pond water cultures. A very interesting project could be done where one finds as many different kinds of micro-organisms as possible. Drawings should be made to scale (see Lab. 2, part (d)) of each organism and such references as those listed in the appendix would be most helpful in their classification. Consult with your teacher for suggestions on how to proceed. It may be wise for one person (or group) to work with the animals while another group works with the plant life.

LAB. 4

UNITS OF PROTOPLASM—PLANT CELLS

Object—to examine onion epidermal cells in order to learn the nature of one kind of plant cell and observe some of the major structures therein.

Materials—microscope, slide—1, cover slip—1, scalpel—1, iodine stain (see Appendix A, 5), piece of an onion bulb.

Procedure—(a) Remove a thick scale of onion and using the scalpel peel a piece of the epidermis from the inner surface. Mount the piece of epidermis on a slide in a drop of water. Add a cover slip and examine the living cells under low power and under high power.

(1) What color is the living protoplasm?

(2) Is there any movement of the cell contents? If so, describe this.

(b) Raise one side of the cover slip and add a drop of iodine stain. This will kill the cells, but will enable you to see their parts more clearly. Examine under

low power. Note especially the cell walls and nuclei. The nuclei appear as yellowish, spherical bodies.

Describe how the cells compare in shape, the number of nuclei present per cell, and any other cell components present.

Draw a group of the cells. Make them the proper size for the magnification used (low power). Show the cell wall, nucleus, and cytoplasm. Label the whole drawing, and record the magnification.

Select one cell which shows the content clearly. Move it to the centre of the microscope field. Examine all parts of the cell under high power. As you study the cell, shift the focus with the fine adjustment and note that the cell has thickness as well as length and width. You should see the cytoplasm filling most of the cell. It is bounded by the plasma membrane. Cavities in the cytoplasm, vacuoles, are each bounded by a vacuolar membrance. The droplets in the cytoplasm are oil droplets. Draw a single cell from the high power field, making it the appropriate size for this magnification. Include the nucleus. Label: plasma membrane, cytoplasm, vacuolar membrane, vacuole, oil droplet, nucleus. Study the nucleus carefully. You should observe one or more darkly stained spots in the nucleoplasm. Each of these spots is a nucleolus. Show them in your drawing.

LAB. 5

UNITS OF PROTOPLASM—ANIMAL CELLS

Object—(a) to examine human epithelial cells in order to learn the nature of one kind of animal cell and observe some of the major structures therein.

(b) to compare the structure of plant and animal cells.

Materials—microscope, slide—1, cover slip—1, clean toothpicks—2, iodine stain.

Procedure—(a) Scrape the inside of your cheek with a clean toothpick. Smear the material on a slide. Add a drop of iodine stain and a cover slip. Examine under low power, noting the masses of cells and individual cells. Move the slide around so that you can observe one cell which does not appear to be folded over or badly distorted from scraping and smearing. (See Modern Biology p. 39.) Observe the cell membrane, cytoplasm, and nucleolus.

Turn to high power and again observe the same cell. Draw the cell and label the parts observed. Make the drawing the proper size.

(b) From your observations of the onion epidermis and the cheek epithelium discuss the fundamental similarities and differences between plant and animal cells.

We have implied that one is able to see the plasma membrane which surrounds the nucleus, vacuoles, and cell cytoplasm. This is probably not true since the light microscope is not capable of resolving objects smaller than 0.0001 mm. One may demonstrate the presence of the plasma membrane by plasmolysis of the cell. Plasmolysis and micro-injection experiments have conclusively demonstrated the existence of the plasma membrane. It forms an essential living part of every cell and is the "screen" through which all substances taken into cells must pass. It is elastic and pliable in some cells, quite rigid in others. It is capable of limited repair if punctured and is also capable of growth as the cell enlarges. The electron microscope shows the membrane to be double. (See Fig. 4-4, page 37, Modern Biology, nuclear membrane.) The total thickness of the membrane is only about ten millionths of a millimeter!

You observed that both types of cells had certain features in common. The reason for this is that all living cells must carry on the process of metabolism in order to survive.

(1) What is meant by the term metabolism?

(2) List the essential metabolic processes common to both types of cells studied.

(3) What functions are served by cell membranes of both cells?

(4) Where, in the cells, is the genetic material located?

(5) Briefly explain the relationship between cells, tissues, organs and systems.

Note re classification: (See Modern Biology, Ch. 8.)

The modern system of classification places a particular organism in one of FOUR kingdoms. Traditional classification used only two kingdoms. This laboratory workbook is organized on the basis of the modern classification scheme.

Kingdom—Monera
- Phylum Schizophyta—Bacteria
- Phylum Cyanophyta—Blue-green Algae

Kingdom—Protista
- Algae
  - Phylum Chlorophyta—Green Algae
  - Phylum Euglenophyta—*Euglena*
- Fungi
  - Phylum Mycophyta—Yeast, bread mold
- Protozoa
  - Phylum Sarcodina—*Amoeba*
  - Phylum Ciliata—*Paramecium*

Kingdom—Plantae (Metaphyta)

Kingdom—Animalia (Metazoa)

LAB. 6

BACTERIA

NOTE: 1. Students should do ONE of the following parts depending upon facilities available.

2. Bacteria cannot be seen under a microscope without an oil immersion lens and suitable staining. (See Appendix A, 5 for one staining technique.) The following exercises have been planned to demonstrate the presence of bacteria, their appearance on culture media, and some of the effects they cause.

Part I: Milk Spoilage: (Work in groups of four)

Object—1. to observe the sequence of micro-organisms supported by milk and the effects of the micro-organisms on the milk.

2. to observe in the decomposition of milk one of the major functions of bacteria—that of releasing raw materials from organic substances making them again available for use in the synthesis of new organisms.

Materials—raw milk—¼ pint per class, pasteurized milk—½ pt. per class, sterile test tubes—4 per group, pH test papers, sterile nonabsorbent plugging cotton—enough to plug the test tubes, test tube rack—1, wax marking pencil—1, incubator—(desirable but not necessary).

Procedure—Half fill two test tubes with raw milk and the other two with pasteurized milk. Mark the tubes so that they may be identified. Check and record the pH of each of the samples. Plug loosely with cotton and incubate at 37° C.

(a) Make daily observation of pH, odor, and appearance of the samples for a week and record your observations. Permit the samples to stand for another two weeks and observe every few days until no further changes appear.

(b) In the decomposition of milk observed above the order in which the different flora appeared was as follows:

i. *Streptococcus lactis* and related bacteria multiply rapidly and produce sufficient lactic acid to decrease the pH to about 4.5, causing curdling. (Test with pH paper.)

ii. *Lactobacillus* species start to multiply below pH 6 and continue the fermentation producing pH 3.0 to 3.5.

iii. Few micro-organisms can grow in a medium of this acidity. Under anaerobic conditions, however, molds and film-producing yeasts can utilize the acid as a source of energy by oxidizing it to CO_2 and H_2O. With the utilization of the lactic acid by the molds and yeasts the medium once more becomes nearly neutral (pH 7.0).

iv. Now other bacteria such as *Pseudomonas* and spore-formers finally digest the casein and fat and reduce the milk to a dirty-looking, watery, putrid or rancid liquid.

(1) From your observations of the above demonstration what can you conclude about the effect of pasteurization on the keeping qualities of milk?

(2) If pasteurization has little or no effect on the keeping qualities of milk, what is the purpose served by this process?

Optional (d) Since *S. lactis* and *Lactobacillus* are active in the souring process of milk, one should expect to find them in great numbers in such foods as cottage cheese, yogurt. If a suitable microscope is available, prepare stained microscope slides of these foods and examine them to confirm this. (See Appendix A for staining procedures.)

Part II: Dye Reduction Test for Bacterial Contamination of Milk

Note: Since most schools will not have a laboratory period long enough for this test to reach its end point during the period, it is suggested that the teacher may set it up as a demonstration. An alternate suggestion would be that one or more students may wish to perform the demonstration for the class. This would mean, of course, that it would have to be set up ahead of time so that the end point would be reached during the regular lab. period.

Object—to determine the degree of bacterial contamination of milk by using the methylene blue reduction test.

Materials—milk samples*, test tubes (20-25ml), plugging cotton, test tube rack—1, incubator desirable, methylene blue dye (see Appendix A, 5).

* Ideally one could test several samples of raw milk from different sources presumably with different degrees of contamination. Pasteurized milk could also be included in the test. However, it is often difficult to obtain these samples so, as an alternative, one could obtain one fairly large sample of milk from any source and deliberately subject portions of the sample to various types and degrees of contamination. Keep some of the sample free of contamination to act as a control.

Procedure—The ability of bacteria to transfer hydrogen to dyes is utilized in the dye reduction test for grading raw milk. Generally speaking and with conditions of incubation kept constant, the greater the number of bacteria, the shorter is the time required to reduce the dye. Methylene blue becomes colorless when reduced by bacterial action. First prepare the dye. Since the concentration of dye in the milk is in the ratio of 1:300,000 the following procedure may be used: Mix 1 c.c. of stock solution of dye (see Appendix) in 300 cc. of clean water. This will dilute the dye to 1 part dye to 30,000 parts water. Use 1 cc. of this dye solution in each of the 10 cc. milk samples and this will give the correct dye concentration. (Use care in measuring all amounts.)

Put 10 cc. of each of the milk samples you wish to test in clean test tubes. Label each tube as 1, 2, 3, etc. Add 1 cc. of methylene blue dye as prepared above to each test tube. Shake, plug, and place in the test tube rack. Incubate at 37° C. and record your observations at half-hour intervals. Use a table as shown below.

The reduction end-point is 80% decoloration of the methylene blue; that is, the upper 20% of the milk may retain the blue color. A milk sample that produces this end-point within one-half hour contains many bacteria, probably millions per cc. Grade A raw milk to be used for pasteurization should not reduce methylene blue in less than five hours.

These tests and demonstrations indicate the necessity of observing sanitary procedures in milking and processing milk in the home or dairy and in the maintenance of the building where the cows are milked. While the organisms mentioned above are not usually pathogenic, pathogens may be present in milk. Such diseases as tuberculosis, Q fever, undulant fever, and septic sore throat may be transmitted via raw milk to the consumer.

Results—Record your results in a table with the following column headings: Use column I of the table to record the time the samples were placed in the incubator. Use column II to indicate the time when the sample first showed noticeable decolorization. Use column III to indicate the time that the test end-point was reached. (20% decolorization.)

Milk Sample	Source of Sample (or Source of Contamination)	I	II	III
Control				
1				
2				
3				

PART III: BACTERIA IN OUR ENVIRONMENT (Work in groups of four with each group testing two areas for bacterial contamination).

Object—to demonstrate that bacteria are present in vast numbers and in great variety throughout our environment.

Materials—sterile swabs in plugged test tubes—2 (in the one tube), test tubes—3. These should be small plugged tubes each containing exactly 4 cc. of water after sterilization. Test tubes of sterile tryptone glucose extract agar—3. (See Appendix A-5.) (Each tube should contain about 15 cc. of culture medium. It should be plugged and sterilized before use.) Sterile pipettes and petri dishes—3 of each, wax marking pencil—1.

Procedure—With the wax marking pencil put "A" on one of the tubes of water, and one of the petri dishes. Put "B" on another set and "C" on the remaining set. Set "A" should be reserved as a control.

(a) Decide where you are going to take your sample (desk top, floor, door knob, drinking fountain, your hand or hair, etc.). Remove one swab from the plugged test tube (replug the tube). Unplug the tube of sterilized water marked "B" and dip the swab into the water. Press and roll the swab against the side of the tube to remove excess water. Swab slowly and firmly three times over the surface to be tested. Rinse the swab in the same tube of sterile water and again swab three times the same surface. Finally cut off the swab with a pair of scissors or break it off short in the mouth of the same tube of water (B) and allow the swab to fall into the water. The tube of water now contains the swabbings and part of the swab stick. Replace the cotton plug firmly and with your thumb over the plug, shake the tube and contents rapidly about fifty times. Repeat this procedure with the second swab and tube "C" of sterile water but test a different site.

(b) Use one of the sterile pipettes to transfer 1 cc. of the water from test tube "B" to petri dish "B". Pour in one tube of culture medium which has been melted in hot water (45° C but no hotter). Mix well by giving the plate a rotating, rocking motion, using care not to allow agar to spill over the rim or splash on the lid. Set aside to cool.

(c) In the same manner but using the other pipette, transfer 1 cc. of the water from the swabbings in tube "C" to petri dish "C". Add the culture medium, mix and set aside to cool.

(d) Transfer 1 cc. of the sterile water from tube "A" to petri dish "A". Add the culture medium, mix and set aside to cool.

When all plates have cooled so that the agar has solidified, invert the plates and incubate them at 35-37° C for 48 hours.

(e) After 48 hours, count the number of colonies in each plate and record your results.

Sample	Area Tested	Number of colonies after 48 hrs.
Control A		
B		
C		

(1) What is the purpose of the control in this experiment?

(2) The results observed probably indicate that our environment contains vast numbers of bacteria and fungi. What explanations can you offer to account for the fact that we rarely become ill from bacterial diseases?

BLUE-GREEN ALGAE

Object—to study the blue-green algae in order to determine: 1. the general nature of plants belonging to this phylum; 2. why they are classified as Monera along with bacteria.

Material—microscope, slide—1, cover slip—1, culture of any blue-green alga, such as *Oscillatoria, Anabaena, Nostoc.* (See Modern Biology, p. 94.)

Procedure—prepare a wet amount of a **small portion** of the culture of blue-green agae. Examine it under low power. By referring to Modern Biology, page 94, try to identify the alga in view. (For further references to assist in identification of algae (see Appendix A, 4).

(1) Describe any movement maintained by the algae.
Isolate (a few) cells of the organism and examine them carefully under high power.

(2) Can you detect the presence of a cell wall around these algal cells?

(3) Is there a gelatinous matrix present around the alga?

(4) Is there a definite nucleus present in any of the cells?

(5) Where is the photosynthetic pigment located in the cells?

(6) What pigment other than chlorophyll is responsible for giving these algae their typical blue-green color?

(7) How do blue-green algae reproduce?

(8) In point form, list the reasons why bacteria and blue-green algae are placed in the same taxonomic Kingdom, the Monera.

DESMIDS—UNICELLULAR ALGAE

Object—to study the structure of one type of unicellular alga.

Materials—microscope, slide—1, cover slip—1, living culture of mixed desmids, (see Appendix A, 2).

Procedure—There are about 24 genera of desmids of the Family Desmidiaceae found in Alberta. The main differences between them are: cell shape, ornamentation of the cell wall, and structure of the chloroplast.

Take a sample of the desmids collected and put a drop of the culture on a slide. Cover with a cover slip and examine under low power.

In the space provided below, make simple drawings of the different desmids found in the culture. By using an appropriate reference try to identify your specimens. (See Appendix A, 4.)

Select one of the desmids for study under high power. Draw the selected desmid to scale. Label the cell wall, nucleus, vacuoles, chloroplasts, and pyrenoids.

Reproduction in the desmids is by cell division (fission), by aplanospores, and sexually by zygotes that are formed by a conjugation type of gametic union.

(1) List four major structural differences that exist between desmids and members of the Phylum Cyanophyta.

FILAMENTOUS GREEN ALGAE

Object—to study the structure and reproduction of a filamentous green alga.

Materials—microscope, slide—1, cover slip—1, forceps—1, appropriate culture of algae (see Appendix A, 2).

Procedure—Prepare a wet mount of several filaments of the alga culture. Do not take too large a mass or you will not be able to isolate a filament for study. Examine the slide under low power. Select one filament that is in good condition and move your slide so that you can examine the filament along its length. Determine if the filament branches at any point. Also be alert for signs of conjugation.

By using low power to locate the different kinds of filamentous green algae present on your slide and high power to study the detail of the cell, make scale drawings of the different genera present. You needn't draw more than one cell of each in detail but show the cell walls of the two adjoining cells next to it. If possible, also identify each genus present.

(1) Describe the chloroplasts found in the plant which you observed.

(2) You have no doubt observed that dried prunes swell when put in water. As a matter of fact, they may absorb so much water by osmosis that the skin of the prune may be ruptured. Green algae manufacture sugars by photosynthesis during the hours of daylight. What explanation can you offer to explain why the alga cells are not ruptured as was the prune?

(3) The size of the aquatic animal population of a pond or slough is often limited by the numbers of aquatic green plants present in the body of water. Give TWO reasons why this is so.

YEASTS AND FERMENTATION

Note—this lab. requires a waiting period of 48 hours after being set up.

Object—to study the structure, nutrition, and reproduction of yeast.

Materials—(work in groups of 4)—yeast suspension—10 cc.[1]*, brown sugar solution—15 cc.[2]*, table sugar solution—15 ml.[2]*, potato water—15 ml.[3]*, honey solution—15ml.[2]*, apple juice—15 ml., corn starch suspension—30 ml.[2]*, iodine stain, test tube rack—1, respirometers—7 (see Appendix A, 5), test tubes—6, Benedict's solution, bunsen burner, wax marking pencil, test tube holders—1, large beaker for waste solutions.

*1. Yeast suspension: mix 1 pkg. of dry yeast in about 200 ml. of water in a beaker. Stir until all yeast is dispersed. This will prepare enough yeast suspension for the class.

*2. Mix about 2 teaspoons of each material in 200 ml. of water to prepare adequate nutrient solutions for the class.

*3. Boil a finely sliced potato in 250 ml. of water for 10 minutes. Allow to cool and pour off the water into a clean beaker. This should be adequate potato water for a class.

Procedure—Number the seven respirometers from 1 to 7. Also number the test tubes 1 to 6. Number the beakers of nutrients as follows:

1.—brown sugar	4.—honey
2.—table sugar	5.—apple juice
3.—potato water	6.—cornstarch

In succession test each of the above nutrient solutions for the presence of simple sugars. To do this, place about 1 ml. of the nutrient solution in a test tube (Solution 1, in test tube 1, etc.). Add 1 ml. of Benedict's solution. Using a test tube holder, gently heat the contents of the tube over the bunsen burner. **(Caution: Do not point the tube at anyone or they may be scalded.)** If a simple sugar is present it will produce a brick red precipitate upon being heated. Record your results in a table with the following data. Indicate whether results were positive or negative.

Tube	Column No.	1	2	3	4
No.	Nutrient	Sugar test Before Yeast Added	Was Gas Evident After 48 Hrs ?	Alcohol Odor After 48 Hrs ?	Sugar Test After 48 Hrs. Fermentation
1	Brown Sugar				
2	Table Sugar				
3	Honey				
4	Potato Water				
5	Cornstarch (Uncooked)				
6	Apple Juice				
7	Cornstarch & Saliva				

Following the instructions in the Appendix A, 5, prepare each of the respirometers. Respirometer No. 6 will be filled with the raw starch suspension and to the remaining 12-15 ml. of starch suspension add 20-30 drops of saliva. Prepare respirometer No. 7 with the starch and saliva mixture. Set all respirometers in the test tube rack and incubate at 37°C for 24 hours. (If no incubator is available, place the tubes in some warm location to incubate.)

After 48 hours examine the respirometers and complete column 2 in the above table.

Open the respirometers one at a time, and complete columns 3 and 4 as you proceed from one respirometer to the other.

Prepare a microscope slide of one of the fermented materials. Stain the yeast with iodine. Examine under low power and try to find yeast cells which are budding.

Examine one budding yeast cell carefully. (Highest power.) Observe: cell wall (not of cellulose as in most plants), nucleus, large vacuole (near nucleus) and there may be granules of carbohydrates present—they stain deep reddish brown with iodine. Draw and label a cell of yeast as seen under high power.

(1) Account for the results demonstrated in columns 1 and 4 of the table.

(2) What form of nutrition is utilized by yeast cells?

(3) In what materials was there no fermentation? Why?

(4) Account for the different reactions in respirometers 5 and 7.

BLACK BREAD MOLD

Note—this lab. requires waiting periods of 24 and 72 hours after being set up.

Object—to study the dispersal, growth habit, and reproduction of a fungus.

Materials—(work in pairs) sterilized culture medium—1 plate (see Appendix A, 5), microscope slide—1, cover slip—1, wax marking pencil—1, permanent slide of conjugating bread mold, microscope.

Procedure—(do not open your petri dish—it has been sterilized)

(a) Decide where you wish to expose your culture plate (classroom, basement, outdoors, lunchroom, etc.). When you have decided upon a location, take your unopened petri dish to the location, open it up and leave it in the location for 15 minutes. After 15 minutes replace the cover and place your plate in the incubator where it will be kept at 35°C for 24 hours. Be sure to mark your plate with the wax marking pencil so that you will be able to identify it. Also record where you exposed it. (Go on to part (b) of this lab.)

Record the number of separate colonies visible on the plate after 24 hours and after 72 hours.

Determine the area of the plate and from the information recorded above, calculate and record the number of spores falling per hour per square foot.

Compare your results with those of other members of your class who exposed their plates in different locations. Record your findings.

(b) Place a drop or two of the culture medium provided on a clean slide. It may be necessary to warm the slide gently to soften the solidified medium and then press a clean cover slip lightly on top. There should be a space of about 1mm. between the cover slip and the slide below it. "Seed" this medium with mold spores by placing it on some moldy bread and blowing across the bread to transfer the spores to the medium. Place this preparation in a covered container where it will not dry out; a bowl with water in the bottom and the slide supported above this. Incubate until mold mycelia and sporangia appear. (Go on to part (c) of this lab.) Remove the slide from the bowl and observe under the microscope; do not remove the cover slip. To prevent drying of the mycelia while viewing, put a ring of vaseline around the outside of the cover slip to fill in the air space between the slip and the slide. Do not force the vaseline under the cover slip. (See Fig. 3.) If you observe the mold for some time under the microscope, you may detect the protoplasmic streaming within the cells of the mycelium. Tap the stage of the microscope and watch closely.

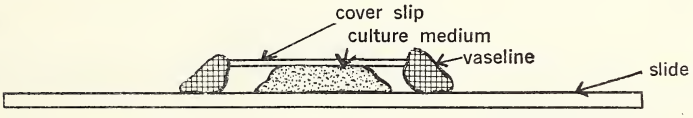


Fig. 3

Make a drawing of a bread mold. Label the following structures: sporangium, sporangiophore, stolon, rhizoid, mycelium.

(c) Examine a permanent slide showing conjugating bread mold under low power of the microscope. The mycelium on this slide is of two kinds, plus and minus.

Find two hyphae growing close together with branches growing out towards one another. In some there will be cells formed at the end of each of these branches (suspensors). These cells are gametes, two of which combine to form a zygote. If the gametes cannot be distinguished in appearance (isogametes), the process is called conjugation. A plus gamete will combine with a minus gamete to form a zygote, but two plus gametes or two minus gametes will not combine. The zygote grows a thick, rough, dark wall around itself to form a zygospore.

Draw plus and minus hyphae showing stages in the formation of the zygospore. Label fully.

(1) What type of reproduction is represented by the conjugation process?

(2) Why is it apparently necessary for this mold to produce zygospores although it produces spores in the sporangia?

LAB. 12

Euglena—A FLAGELLATE ORGANISM

Object—to observe the structure and activities of *Euglena.*

Materials—culture of *Euglena* (see Appendix A, 2), microscope, slide—1, cover slip—1, medicine dropper—1.

Procedure—*Euglena* is usually found in fresh water ponds and slow-moving streams high in nitrogenous compounds. In cultures, it makes up part of the greenish scum on top of the water or along the side exposed to sunlight. Take a drop of the water with a dropper and put it on a clean slide. Put a cover slip in place and examine the slide under low power. The small cigar-shaped *Euglena* will be seen swimming rapidly on the slide. After their movements have slowed, observe them under high power. (It may be necessary to add a drop of methyl cellulose to the culture on the slide in order to slow the *Euglena* so that it may be observed under high power.)

(a) Locate the following structures: flagellum (use dim light), nucleus, chloroplast, gullet, reservoir, eye spot, pellicle, paramylon food storage structures (rods, plates, rings, or discs).

Make a scale drawing of the organism and label all parts.

(1) What is the color in the cell and how is the color distributed?

(2) How does the cell move and in what direction?

(3) What is the function of the eye spot?

(b) See if you can find an animal moving by drawing the posterior part of the cell forward to become rounded, then extending its anterior end to push forward. This type of movement is called euglenoid.

LAB. 13

Amoeba—A PROTOZOAN

Object—to identify the structural parts of *Amoeba,* and observe their action and the behavior of the animal.

Materials—culture of living *Amoeba* (see Appendix A, 2), prepared slide of *Amoeba,* microscope slide—1, cover slip—1, eye dropper—1, microscope, acetic acid or vinegar.

Procedure—place a drop of culture on a clean slide and lower a cover slip on to it. Examine under low power with dim light until you find an *Amoeba.* Specimens of *Amoeba* appear as irregular-shaped, grayish-colored particles. Make a scale drawing

of an *Amoeba* and label all of the following parts that can be identified: plasma membrane, ectoplasm, endoplasm, pseudopodium, cytoplasm, nucleus, food vacuole, contractile vacuole.

Observe the organism under high power and note the streaming of the cytoplasm.

(1) How does the *Amoeba* move from one place to another?
(2) There are no openings into the cell. How does the animal obtain its food?
(3) Would you class this cell as highly specialized? Why?
(4) Place a drop of dilute acetic acid at the edge of the cover slip. Observe and record the reaction of the *Amoeba.*

LAB. 14

Paramecium—A CILIATE PROTOZOAN

Object—to observe the structure and activities of *Paramecium.*

Materials—culture of *Paramecium* (see Appendix A, 2), medicine dropper—1, slide—1, cover slip—1, microscope, fine cotton fibers or methyl cellulose.

Procedure—*Paramecium* is very abundant in stagnant water where it is often found in the scum which forms on the surface. Pick up a drop of scum from a *Paramecium* culture and place it on a clean slide. Without a cover slip, examine it under low power. You will see these animals darting about rapidly. Now put a few cotton fibers on the slide to form small traps, or use a solution of methyl cellulose, and mix a drop of this solution with the paramecia on the slide. Place a cover slip on top and examine again under low power.

Make a scale drawing of *Paramecium* and label all the structures.

(1) How do *Paramecium* react when they bump into an obstacle? Locate an animal that is not swimming. Center it in the field and switch to high power. Refer to Modern Biology, page 234 and try to identify the structures shown in Fig. 20-7.
(2) How can you distinguish between the anterior and posterior ends?
(3) Examine the *Paramecium* carefully using very dim light. (Adjust the substage diaphragm.) What are the short hair-like structures on the outside of the cell and for what are they used?
(4) *Amoeba* and *Paramecium* both belong to the Protozoa but obviously differ structurally in a number of ways. List the structures present in *Paramecium* which were not present in *Amoeba.*

LAB. 15

THE BRYOPHYTA

Object—to study a relatively simple plant, become acquainted with its structure, and its life cycle.

Materials—living specimens of mosses and liverworts (see Appendix A, 2), hand lens.

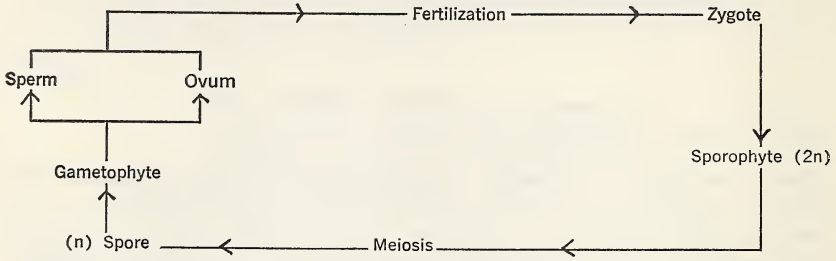
Procedure—refer to your text, Modern Biology, pages 127-130. Obtain a specimen of moss or liverworts from your teacher and observe: (you may wish to use a hand lens for examination of detail).

1. The sexual reproductive structures
2. The green gametophyte

3. The rhizoids
4. Gemmae (asexual) if present
5. The brownish sporophyte (if present).

Make a drawing of a male and a female liverwort plant and label the above structures.

The bryophytes exhibit a true alternation of generations in their life cycles. An independent, green, haploid, sexual, conspicuous gametophyte generation alternates with a parasitic, diploid, asexual, inconspicuous sporophyte generation.



Draw male and female moss gametophytes, show an enlargement of the antheridium and archegonium. Also draw a complete moss plant showing the gametophyte with the attached sporophyte.

(1) How is fertilization accomplished (a) in mosses? (b) in liverworts?
(2) The gametophyte generation and the gametes produced by the gametophytes are haploid. By what process are the gametes produced?
(3) By what process are the spores produced by the sporophyte generation?
(4) Give at least two good reasons why bryophytes must live in a very moist environment.

Project suggestion: culturing moss protonema—see Morholt, Appendix A, 4.

LAB. 16

FERNS

Object—to study the gross structure of a fern and compare its life cycle with that a bryophyte. (See Modern Biology pp. 130-132.)

Materials—fern specimen (sporophyte) (see Appendix A, 2), hand lens, preserved fern gametophytes.

Procedure—examine the specimen carefully. Observe that the frond, a compound leaf, has several leaflets attached. Note the veins in the leaflets and there may be brown sori on the under surfaces. Examine them with a hand lens. The stalk of the leaf, the petiole, is attached to an underground stem or rhizome. Note the true roots arising from the rhizome. This plant represents the sporophyte generation. Draw a mature fern sporophyte and label the above structures.

(1) Compare the fern sporophyte with that of the moss.
(2) From what structure would the gametophyte generation arise?
(3) Examine the under surface of a fern prothallus with a hand lens. Note the antheridia and archegonia, rhizoids, apical notch. Draw the gametophyte as seen from the under surface and label the above structures.
(4) The fern gametophyte is haploid but the sporophyte is diploid. Explain where and by what processes these conditions come about.

ROOTS—EXTERNAL FEATURES

Object—to examine the various regions of roots and learn their functions.

Materials—four-day-old grass seedlings (see Appendix A, 2), radish seedlings, microscope, slides—2, cover slips—2, 50 ml. beaker of water—1, medicine dropper—1.

Procedure—as with all of the other vegetative organs of the plant, the roots show much variation. Remember that roots cannot be characterized by where they grow but rather by internal structure. So that you may appreciate the origin of the parts, the root will be studied from a developmental standpoint.

The root tip. A four-day-old grass seedling, such as red top or timothy, which has been grown on water, will be provided. After placing the root tip in water on a slide, cut off the next half inch from the root and discard it. Be sure there is water surrounding the tip; do not press down on cover slip. Keep the root tip immersed in water constantly while studying it. Study the root tip externally under low power, beginning at the tip end. Covering the tip is a root cap made up of a rather loose mass of cells. While roots which are grown in water show a loosely textured cap, a similar root grown in soil would have a more restricted one. In the soil some of the cells would be ground up and left along the side of the root. This root cap normally protects the rest of the young root as the tip is forced through the soil. Behind the cap, there is a smooth region in which the cells are small and dark. This is the apical meristem and is the region of cell division. This apical meristem forms not only new cells within itself but the root cap as well. The addition of the new cells accounts for some of the increase in length of the root. Behind the region of division, the cells begin to elongate and may show a slight differentiation. This is the region of elongation and is responsible for most of the increase in length of the root. Behind this, is the region of differentiation or the root hair region. While the cells are differentiating internally, the root hairs form externally, thus establishing the great absorbing center of the root. Farther back in the older part of the root, all the cells are mature and the primary development of the root is complete. These regions have no exact lines of demarcation separating them; they merge into one another. Compare the regions of the root tip of the grass seedling with those of the radish seedling.

Make a labelled drawing showing the regions of the root.

As the elongation of the root is completed, many of the epidermal cells form root hairs. They appear at first as small papillae which gradually grow in length at their tips. Each root hair is a lateral tubular extension of an epidermal cell. The mature root hair consists of cell wall, plasma membrane bounding a thin layer of cytoplasm, and a vacuole with cell sap; all of these are continuous with the same structure in the body of the cell. In some, the nucleus may be seen near the tip of the root hair as a gray body. Cutting down the amount of light and careful focusing under high power may reveal the streaming of the cytoplasm in root hairs. The functioning root hairs make up the bulk of the external surface of the root hair region. Along the oldest part of the region, the root hairs become altered, wither, and gradually disappear. Through the functioning root hairs, much of the water and water soluble materials are absorbed for plant use.

Make a large labelled drawing of one complete epidermal cell with root hair and adjacent epidermal cells.

ROOTS—INTERNAL STRUCTURES

Object: (1) to examine prepared slides of monocotyledonous and dicotyledonous roots in order to determine the arrangement of the tissues of the maturation region;

(2) to compare adventitious with ordinary roots as to points of origin.

Materials—prepared slide of monocot. and dicot. root cross sections, microscope, cutting of geranium, coleus, or any other plant. (The cuttings should be rooted in water so that the roots are visible. Those which have just started to root are best.)

Procedure—*Note:* detailed instructions are provided for examination and study of the dicot. root section only. The same general procedure should be followed in examining the monocot. root section. You should note in the monocot. root, the absence of one type of tissue that was present in the dicot. and also that the arrangement of the tissues of the central cylinder region is markedly different.

Cross section of a young dicot. root. Select a slide bearing a stained cross section of a root cut just behind the root hair region. In this region, all of the tissues which are derived directly from the apical meristem have differentiated. These are primary tissues. In later development through the activity of the vascular cambium and other cambia, other tissues may be formed. These would be secondary. Examine with both low and high power, starting from the outside of the section and working toward the center.

The epidermis is the outer layer of cells. Directly underneath is the relatively wide layer of thin-walled cells constituting the cortex. This layer of parenchyma cells extends completely around the root and most of the cells have conspicuous contents.

(1) What is the main function of the cortex?

The innermost layer of cortex is the endodermis, one cell in thickness and with many of the cells showing thick walls. The pericycle lies just underneath the endodermis and is one or two cells in thickness. The cells are larger than those of the endodermis. In the center of the section, there is a solid core of thick-walled, heavily stained xylem cells. In this cross sectional view, the core of xylem tissue shows four radiating arms, each one coming in contact with the pericycle. The phloem tissue lies, like a series of tiny islands, between the xylem arms and is composed of small cells with thin walls, usually with stained contents. Between the xylem and phloem, there may be a region of cells showing evidence of division. This strip of cells is the cambium and one of the middle tangential rows of cells would be the active vascular cambium. At this stage of development, most of the sections will show only weakly developed cambia confined to the regions between the xylem arms. Later these individual vascular cambia extend laterally and join to encircle the root.

Before examining the monocot. root section, make a labelled drawing of the dicot. root section as seen under low power. When you have completed this drawing, study the monocot. root section in a similar manner and make a labelled drawing of this section as seen under low power.

(2) What tissue was present in the dicot. root that was lacking in the monocot. root?

(3) Carefully describe how the arrangement of the xylem differed in the two root sections studied.

Branch or secondary roots arise in the pericycle and will have the same structure as the primary root. The term secondary as used here applies only to the origin of the root; each root may or may not have secondary tissues.

Examine the plant cutting with the short roots growing on it. These roots are classed as adventitious roots because they have originated from a region where one would normally not find roots—specifically from a portion of the stem, the node. Slips or cuttings are used for asexual propagation of many plants.

(4) Give examples of other plants that normally develop adventitious roots even though the plant has not been cut.

LAB. 19

DIFFUSION AND OSMOSIS

It is suggested that the class be divided into two sections with Section I working on diffusion and Section II working on osmosis.

Object—1. To study the phenomenon of diffusion in air and water;
2. To observe the movement of water through a semi-permeable membrane by the process of osmosis.

Material—(work in groups of four).

A. Diffusion

diffusion tube—see Appendix A, 5
ammonium hydroxide (conc.)
watch with second hand
250 ml. beaker—1
medicine droppers—2
methylene blue dye (from stock)
wax marking pencil—1

B. Osmosis

dialysis chamber (see Appendix A, 5)
5 mm. x 4 ft. glass tubing—1
ring stand—1
burrette clamps—2
1-hole #12 corks—2
glucose solution—15 ml.
rubber bulb syringe—1
Benedict's solution
test tube holder—1
bunsen burner

Procedure:

A. **Diffusion** (work in groups of four).

(a) Diffusion in air.

Place the prepared diffusion tube (Fig. 9) in a level position. Use a medicine dropper to moisten the plug of absorbent cotton with concentrated ammonium hydroxide solution. Observe and record the time required for the NH_4OH fumes to affect the color of the phenolphthalein.

Calculate the average rate of diffusion in inches per minute.

(b) Diffusion in water.

Put about 200 ml. of water in the 250 ml. beaker and place the beaker in a location where it will not be disturbed for 24 hours. (Mark your beaker so that you will recognize it.) With the medicine dropper get one or two drops of the dye solution provided by the teacher. (Hold a towel under the dropper so that it will not drip on the floor.) Place the tip of the dropper near the bottom of the beaker and gently squeeze the dye into the water. Slowly withdraw the dropper and wash it out immediately. Record the time and check at the end of the period, after school, and next period to determine approximately how long it takes for an even distribution of the dye in the water.

(1) Define diffusion.
(2) What causes diffusion?
(3) Which appears to be faster, diffusion of a gas in a gas or diffusion of a solid in a liquid? What factors might account for this difference in rate of diffusion?

B. **Osmosis:**

Assemble the apparatus as shown in the diagram. Use the rubber bulb syringe to fill the dialysis chamber with the colored glucose solution. Be sure there is no air trapped in the chamber. Use care when inserting the long glass tubing into the rubber stopper of the dialysis chamber. When you have assembled the tube and dialysis chamber, carefully rinse off any of the sugar solution that may be on the outside of the apparatus. Assemble the apparatus as shown in Fig. 4 and note the level of the sugar solution in the tube. Mark it with a wax marking pencil or a piece of string tied around the tube.

Test about 2 ml. of the water in the beaker with Benedict's solution to determine whether or not any sugar is present in this water.

Leave the apparatus set up for 24 hours and again test the water in the beaker for the presence of glucose. Record your results as positive or negative.

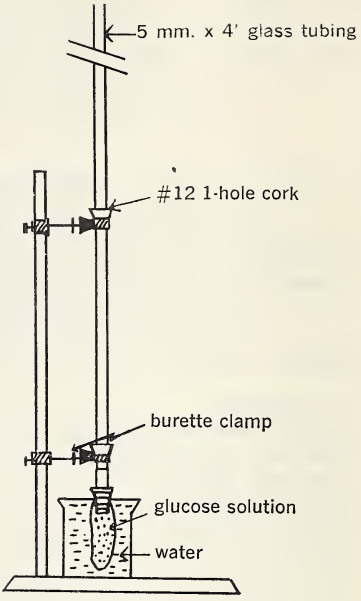


Fig. 4

(1) Does the sugar diffuse out of the dialysis chamber? Why?

(2) Define osmosis.

(3) By using the information gained from this experiment, explain the movement of water from the soil into the root hair of a plant.

(4) How does the water get from the root hair into the water-conducting xylem tissue of the root?

(5) Which moved faster, the sugar molecules out of the chamber or the water molecules into the chamber?

(6) Account for this difference in rate of movement.

LAB. 20

TYPES OF STEMS

Object—to compare the structure of stems of the following types: woody dicotyledon, herbaceous dicotyledon, monocotyledon.

Materials—section of tree trunk (see Appendix A, 5), pieces of herbaceous dicot. stem—(alfalfa, pigweed, bean, sunflower, or any other suitable plant), pieces of monocot. stem—(corn stalks, iris, or gladiolus peduncles), prepared slides of woody dicot., herbaceous dicot., and monocot. stem cross sections, microscope, hand lens.

Procedure—(work in groups of two). (Different groups should work at different activities to avoid overcrowding around wood samples.)

A. Carefully examine the prepared sections of the tree trunks. You should be able to identify the following: Use a hand lens where necessary.

1. Bark—consists of the outer cork, cork cambium, cortex and phloem.

2. Vascular cambium—separates the xylem and phloem regions.

3. Wood—consists of the xylem cells. Note the medullary rays, spring wood, summer wood, and annual rings. You might also note the division into sapwood and heartwood.

4. Pith—soft and usually brown region of compressed parenchymous cells at the centre of the section.
   Examine a prepared slide of a woody dicot. and study the detail of the above regions. Make a labelled drawing of a cross section of the woody dicot. stem.

B. Examine a portion of an herbaceous dicot. stem. Use a hand lens and identify the following regions in the cross section: epidermis, cortex, fibrovascular bundles, pith region. Also examine a prepared slide of an herbaceous dicot. stem in cross section. Make a labelled drawing (a) of the section to show the arrangement of the tissues, (b) of one vascular bundle as seen under high power. Show the cellular detail of the bundle.

C. Examine a portion of a monocot. stem. Use a hand lens and identify the following regions: rind, fibrovascular bundles, pith. Also examine a prepared slide of a monocot. stem in cross section. Make a labelled drawing:

(a) of the section to show the arrangement of the tissues,

(b) of one bundle as seen under high power. Show the cellular detail of the bundle.

   (1) Name four tissues found in the bark region of a woody stem and describe the function of each.

   (2) What structural features serve to distinguish the stems of the monocotyledons and herbaceous dicotyledons?

LAB. 21

EXTERNAL FEATURES OF A DORMANT TWIG

Object—to study the external structure of a dormant woody twig.

Materials—winter twigs (two or three different kinds if possible). (Those of the poplars, *Populus tremuloides, P. Sargentii, P. balsamifera,* and of the common lilac *Syringa* are good for this purpose), hand lens.

Procedure—Examine the twigs closely with a hand lens. Find the terminal bud and the bud scales covering the growing point within the bud. Leaf scars are situated at the nodes. The space between two nodes is an internode. The tiny marks within the leaf scar are bundle scars. Notice the number and arrangement of these. The lateral buds situated above the leaf scars are axillary buds. Rings encircling the twig some distance below the present terminal bud are bud - scale scars, left where the scales of the terminal bud of the previous year were attached. The twig extending from the terminal bud to the first bud scale scars marks the growth of the previous season. You can find the growth of the season before last between the first and second bud scars, and so on. You should be able to determine the age of the twig. Small raised dots, especially on the younger bark, are called lenticels.

With a hand lens examine the two other twigs you collected. Notice the number of leaf scars at a node, the shape of a leaf scar, the number and arrangement of bundle scars, and other characteristics of the two twigs.

Examine the lenticels. Notice how the tissue in the lenticel seems to have grown rapidly, thus causing a break in the bark.

Draw one of the twigs showing its structure as accurately as you can. Turn the twig so that a leaf scar is towards you. State the kind of twig.

LAB. 22

LEAF STRUCTURE

Object—to study the structure of a typical angiosperm leaf and learn the function of its parts.

Materials—Geranium leaf—1, microscope, slide—1, cover slip—1, forceps—1, scalpel—1, prepared slide of leaf cross section.

Procedure:

A. Leaf epidermis—surface view.

Put a drop of water on the microscope slide. Hold the leaf with the underside out stretched over your forefinger. Scrape the surface carefully with a sharp scalpel so as to loosen a bit of the epidermis. Catch the loose edge with forceps and strip off a piece of the epidermis. It is colorless. Make a wet mount of this, taking care that it does not double over. Examine it under low power. Notice that most of the cells are colorless and irregular in shape. These are epidermal cells. Each is surrounded by a wall and contains cytoplasm and a nucleus. You will also observe dark areas in the epidermis. Each dark area is actually a tiny opening between two bean-shaped guard cells. The opening is called a stoma. The guard cells contain chloroplasts.

Make a labelled drawing of the two guard cells and the epidermal cells adjacent to them.

(1) Are chloroplasts present in the epidermal cells?
(2) Calculate the number of stomata per square centimeter.
(3) What is the function of the stomata?
(4) What is the function of the guard cells?

B. Leaf cross section.

Examine carefully under low power a prepared slide of a cross section of a leaf. You should observe the following structures: cuticle, upper and lower epidermis, palisade layer, spongy parenchyma with air spaces between, vein. Observe the vein carefully. Notice the thick-walled cells in the upper half. These are xylem cells. The thin-walled cells in the lower part of the vein are phloem cells. The large rectangular ones are sieve tubes and the small ones between them with prominent nuclei are companion cells. The vein may be surrounded by a sheath of round cells. Between the vein and the epidermal layers there may be thick-walled strengthening cells.

Draw a labelled cross section of the leaf which you studied.

(1) What is the function of the cuticle?
(2) What is the function of the xylem in the vein?
(3) What is the function of the phloem?
(4) Why does the leaf have so many air spaces in the spongy parenchyma?
(5) What is the main force responsible for gas exchange between leaves and the atmosphere?

PHOTOSYNTHESIS

Note: This lab. requires attention on two consecutive days. It is suggested that the class be divided into two groups with one group working on Part A while the other does Part B.

Object—

A. to demonstrate that light is necessary for photosynthesis.

B. to observe evidence of gas exchange between a green plant and its environment.

Materials: (Work in groups of four).

A.

potted geraniums
aluminum foil—6″ squares
light source—200 watt
starch test equipment
(see Appendix A, 5)

B.

water plants *Elodea,* hornwort, etc. or filamentous algae
#17 test tubes (25 x 150 mm.)—4
#5 solid rubber stoppers—4
brom-thymol blue stock solution—4 ml. (see Appendix A, 5)
glass blow pipe 12-18″ long—1
wax marking pencil—1.

Procedure:

A. Potted plants.

Keep all plants in a dark room for 24 hours before starting this experiment. Bring the plants into the laboratory at the beginning of the period and immediately wrap the desired number of leaves in aluminum foil. To do this, fold the piece of foil in half then place it so that the leaf is between the foil. Carefully fold the edges of the foil so that no light can get at the enclosed leaf at any point. Do not damage the leaf. Leave at least half the leaves of any one plant exposed. Place the plants in strong light. It may be necessary (and is usually advisable) to arrange artificial lighting and leave the plants exposed to the light source overnight.

During the balance of the period, get the equipment ready for testing the leaves for starch (see Appendix A, 5).

Next day remove one exposed and one wrapped leaf from the plant. (One pair of leaves per group of four.) Leave the petiole on the exposed leaf and cut this short on the wrapped leaf. This will enable you to identify the leaves during the test.

Test both leaves for starch and interpret the results.

B. Water plants.

Number four test tubes. Fill them about ¾ full with tap water. Add 1 ml. of bromo-thymol blue stock solution. Using the blow pipe, blow into the liquid until it just turns yellow. Add tap water, one drop at a time, to turn the indicator greenish. (The liquid in all four tubes should be approximately the same color.) This establishes the neutral point for this indicator.

Into tubes No. 1 and No. 2 place a 4-6 inch shoot of *Elodea* or a comparable amount of any other suitable water plant. (Do not pack the plants tightly into the tubes.) Tightly stopper all tubes.

Place tubes No. 1 and No. 3 in the dark and the other two in a strong light. Observe at intervals during the period and record your observations. If no change is observed during this time, keep the tubes under the same conditions until the next laboratory period.

(1) Why did blowing into the liquid cause a color change in the dye solution?

(2) Account for any color change in tubes No. 1 and No. 2.

(3) What is the purpose of using tubes No. 3 and No. 4? Explain fully.

LAB. 24

THE FLOWER

Object—to study the gross structure of a flower and determine the functions of the parts.

Materials—lily or some other suitable monocot. flower, rose or some other suitable dicot. flower.

Procedure—examine both types of flowers and locate the following structures: prepare a chart recording the numbers of each which you find in each flower.

The peduncle—the stalk of the flower.
The receptacle—the apex of the peduncle from which the flower parts grow.
The sepals—the outer leaf-like parts, collectively called the calyx.
The petals—the inner leaf-like parts, collectively called the corolla.
The stamens—each consists of a filament and an anther which produces the pollen.
The pistil—the centre part made up of an enlarged base, the ovary, an elongated part, the style, and the stigma at the top. The ovary contains ovules, each of which may develop into a seed.

1. Study your text, Modern Biology, pages 207-208 and in point form outline in detail:
   (a) The development of the male gametophyte from a pollen grain or microspore;
   (b) The development of the female gametophyte from a megaspore.
2. Compare and contrast the degree of development of the gametophyte and sporophyte generations starting with the Bryophyta and ending with the Angiospermae.

LAB. 25

THE SEED AND THE SEEDLING

Object—to study the structure of monocot. and dicot. seeds and the seedlings of each type of plant.

Materials—soaked bean seeds, soaked corn seeds, dissecting needle, bean seedlings, (see Appendix A, 2), corn seedlings (see Appendix A, 2), hand lens, scalpel or razor blade.

Procedure—The embryo sporophyte lies within the ripened ovule or seed. This embryonic plant cannot begin to grow independently until it becomes equipped with a fairly well-developed root, stem, and leaf system. Before these organs have developed sufficiently, the young plant or seedling is dependent for its growth upon organic materials which were deposited in the seed by the parent plant. In some seeds the reserves of organic food substances are lodged chiefly in the endosperm,

in others in the cotyledons. As a seedling grows, the reserve food substances pass over to the growing organs and consequently the endosperm, if present, and the cotyledon or cotyledons, become shrunken and wasted. Finally these structures drop away and the young organism can no longer be called a seedling. It is a young independent sporophyte.

The cotyledons, or embryonic storage leaves are part of the embryo, since they arise from the fertilized egg. It should be remembered that in the Monocotyledoneae, the embryo has only one cotyledon, whereas, in the Dicotyledoneae, the embryo has two.

Part A.—A Dicotyledon.

(a) The bean seed.

Examine one of the soaked seeds of the bean. Note the hilum. This scar lies at the centre of the concave margin of the seed. It marks the point where the seed broke away from its attachment to the wall of the ovule case. Find the micropyle. This small hole is near one end of the hilum. It leads through the seed coat to the embryo. In the ovule this hole served to admit the pollen tube to the female gametophyte. Also observe that the hypocotyl can be seen through the seed coat. It appears as a finger-like swelling, the point of which lies in contact with the micropyle.

With a needle, remove the seed coat, or testa noting its texture. This operation exposes the embryo, for in the bean, no endosperm is present in the mature seed. In other words the embryo and cotyledons occupy all of the space within the seed coat.

Study the embryo. Identify the two large cotyledons. These embryonic storage leaves constitute the two halves of the bean. Between the cotyledons, along the concave border near the micropyle, lies the small body of the embryo. To expose this it is necessary to separate the cotyledons. This operation breaks the connection between the body and at least one of the cotyledons. Examine the body of the embryo closely. Identify the point at which the cotyledon was broken away. This point serves to divide the body into two parts. The rod-like part which lies below the attachment point of the cotyledons is termed the hypocotyl. The epicotyl which lies above the origin of the cotyledons, consists of two parts—the plumule, a pair of embryonic true leaves, and the embryonic bud, a very small mass of growth tissue which is covered by the plumule.

In the bean, the hypocotyl gives rise to all of the roots and to the lower part of the stem. In many plants, as for example peas, only the roots arise from the hypocotyl. When this is so, the cotyledons remain underground. Make a drawing of the internal structure of a bean seed. Label fully.

(b) The bean seedling.

Examine the bean seedling closely. Note the terminal bud, first true leaves, epicotyl, cotyledons, hypocotyl, primary root, secondary roots. Make a drawing of the bean seedling and label the above structures.

(1) What function is served by the cotyledons during the development of the embryo?

(2) How does the length of the root mass compare with that of the stem?

Part B.—A Monocotyledon.

(a) The corn seed.

Strip away the thin transparent covering of a soaked corn kernel. Remember that the outer layers of this covering are derived from the ovule case, whereas the inner layers form the testa of the seed itself.

Examine the naked grain. Identify the embryo which appears as a lighter area on one of the two broad surfaces of the grain. Also notice the silk scar. With a razor blade or scalpel cut a section vertically through the embryo. Study the cut surfaces, using a needle as a probe. Differentiate between the embryo and the endosperm which surrounds it. Also differeniate between the parts of the embryo. The short, stout hypocotyl is directed toward the pointed end of the grain. The tip of the hypocotyl is the radicle and this is enclosed by a protective sheath. Opposite the hypocotyl is the plumule and its protective sheath. The base of the plumule is the epicotyl. The single cotyledon is attached to the axis of the embryo between the hypocotyl and the plumule. It more or less surrounds the axis and lies in contact with the endosperm. Make a labelled drawing of the structure of a corn seed.

(b) The corn seedling.

Examine a corn seedling. Note that the hypocotyl has given rise to all of the true roots, whereas the shoot, which gives rise to all the upper parts of the plant, has developed from the epicotyl.

In older corn seedlings the epicotyl also gives rise to the adventitious prop roots which are supporting rather than absorbing structures.

Make a drawing of the corn seedling. Label the true roots, prop roots, plumule sheath (coleoptile), plumule.

(1) What is a seed?

(2) Starch as such cannot be absorbed by the cells of the developing embryo yet much of the food stored in the cotyledons and endosperm of a seed is starch. Explain how the developing embryo is able to utilize this material as food.

LAB. 26

HYDRA

Object—to study the parts and reactions of *Hydra,* a representative of the Coelenterata.

Materials—culture of living *Hydra* (see Appendix A, 2), prepared slides showing whole mount and C.S. of *Hydra,* hand lens, dissecting microscope, microscope, depression slide—1, cover slip—1, medicine dropper—1, vinegar—50% solution.

Procedure—A. Prepared slide.

Examine the whole mount of *Hydra* under low power. Identify the tentacles, nematocysts, mouth, body stalk, bud, gastrovascular cavity, basal disk, and gonads if present.

Also examine the cross-sectional view and notice the endoderm, mesogloea, and ectoderm. Make labelled scale drawings of the whole mount and the cross-section.

B. Living *Hydra*

By using a medicine dropper, obtain a living *Hydra* from the culture provided. Place it in the depression of a cavity slide. Cover with a cover slip and examine under a dissecting microscope. Observe all of the major body structures.

Place the slide under low pressure of a micrscope and examine the tentacles. Add one drop of dilute vinegar to the edge of the cover slip and draw it under by using a piece of filter paper or blotter at the opposite edge. Observe the discharging of the nematocysts.

Observe a living *Hydra* culture and notice how they feed.

(1) How does *Hydra* capture and eat its prey?

(2) What general body characteristics of *Hydra* make it a member of the phylum Coelenterata?

LAB. 27

PLATYHELMINTHES—THE FLATWORMS

Object—to study two common flatworms and to compare the morphology and life of the free-living one with that of the parasitic one.

Materials—microscope, prepared slides of a planarian (W.M. and C.S.) and tapeworm (W.M.), living planarian (see Appendix A, 2), Petri dish—1, hand lens—1, pipette—1, dissecting needle, preserved tapeworm.

Procedure:

A. Planarian—Make two outline drawings. On one of these label: anterior end, posterior end, right side, head, auricles, eyespots. On the second, label: pharynx, mouth, gastrovascular cavity, brain, lateral nerves, transverse nerves.

Examine a prepared slide of a cross section of a planarian. Observe the dorsal and ventral surfaces, gastrovascular cavity, endoderm, mesoderm, ectoderm, nerves, cilia. Draw and label the above structures in a cross sectional view.

Examine a live planarian with a hand lens. How does the animal move? Make a current of water in the dish with a pipette. How do the animals react? Devise some means of testing the reaction of planarians to light. Record the procedure used and the results. On the side of the head are small extensions, the auricles. Touch the animal in various places with a dissecting needle. Describe the responses.

B. Tapeworm *(Taenia)*

Examine a preserved tapeworm and a prepared slide of the animal. Draw the anterior region showing some detail of the head and several proglottids.

(1) How does a tapeworm get into the human body?

(2) What precaution can people take to avoid becoming infected with tapeworms?

Review—Prepare a table to show a comparison between the planarian and tapeworm with respect to nutrition, digestion, absorption of nutrients, CO_2 and O_2 exchange.

LAB. 28.

EARTHWORM

Note—This laboratory exercise is divided into two sections. Each of the two sections will require approximately one period and the same materials will be required for each.

Object—to study the earthworm in detail. Special attention will be directed towards the study of:

Part A. External features and digestive system.

Part B. Circulatory, reproductive, excretory, and nervous systems.

Materials—preserved earthworm—1, hand lens, dissecting equipment, prepared slide of cross sections through intestine.

Procedure:

Note: As you dissect your specimen observe carefully the relative positions of the various organs. You will be required to draw them as seen in dorsal view and in sagittal view.

Part A: External features and digestive system. Rinse the worm in water and place it in the dissecting tray.

The animal is made up of segments. The anterior knob-like structure is the prostomium. It is not considered a segment. The first segment follows it.

(1) How many segments are there? Segments 31-37 are enlarged to form the clitellum. It secretes the material of the egg capsule.

(2) Run your finger along the ventral surface of the worm. The bristle-like structures that you feel are the setae. How many are there on each segment?

Try to see them with a hand lens. What is their arrangement?

There are several openings in the worm. Locate the mouth between the prostomium and the first segment; a pair of openings of the sperm ducts in segment 15, a pair of openings of the oviducts in segment 14. There is also a pair of nephridiopores in each segment but these are too small to be seen with the hand lens. There are also tiny openings into the sperm receptacles in the grooves between segments 9 and 10, and between 10 and 11.

(3) To what phylum does the earthworm belong? What characteristics distinguish animals of this phylum from other animals?

Put the worm in the tray dorsal side up. Pin the worm securely at a point about three inches posterior to the clitellum. Slant the pin at about 45 degrees so that it will not be in your way. With the dissecting scissors, starting at a point about one inch posterior to the clitellum, make a longitudinal cut through the body wall along the median line. Do not cut into the organs below. Extend the incision from the point of entry to the prostomium.

Spread the cut edges carefully and notice the septa, thin partitions that join the body wall to the gut. Cut these with a dissecting needle and pin the wall back with pins on each side about an inch apart.

Observe the following structures: pharynx—a muscular structure in segments 1 and 5; esophagus, a thin-walled tube in segments 6 to 13; crop—a thin-walled enlargement in segments 14 to 16; gizzard—a thick-walled enlargement in segments 17 to 19; intestine—a dark, thin-walled straight tube through the remainder of the segments to the anus.

(4) What is the function of each of the following:
   (a) pharynx?
   (b) esophagus?
   (c) crop?
   (d) gizzard?
   (e) intestine?

(5) What is the food of the earthworm?

(6) Make an outline drawing of the earthworm and show the digestive system as observed in dorsal view. Number the segments. Show each structure in the correct segment and label fully. Other structures will be added later. Also draw the digestive system in sagittal section.

Part B: Circulatory, reproductive, excretory, and nervous systems.

Using the same worm as in Part A, locate the following structures:

Circulatory System.

Dorsal blood vessel—this runs alng the median line of the gut. It receives branches from the wall of the intestine and carries the blood forward.

Pseudohearts—five pairs of branches of the dorsal blood vessel which encircle the esophagus in segments 7, 8, 9, 10, 11. The blood flows from the dorsal blood vessel ventrally to the ventral blood vessel.

Show the dorsal blood vessel and upper portions of the pseudohearts on your drawing.

Find the ventral blood vessel—this runs along the median ventral line of the gut. The hearts empty into it, and it gives off branches to the wall of the intestine. The blood flows toward the back (posteriorly) in this vessel.

Find the subneural blood vessel — a fine blood vessel running along the median ventral side of the nerve cord. It may be seen by lifting the whitish nerve cord below the gut.

Make a drawing of a sagittal section to illustrate the above structures and label it fully.

Excretory System.

The earthworm has a true body cavity or coelom between the body wall and the gut. It also has a digestive cavity or enteron. The solid residues from the digestive cavity are passed out of the anus as "droppings". The liquid wastes containing soluble materials from the body cavity are removed by the structures listed below which can be seen best in segments along the intestine.

Nephridia—a pair of long coiled tubules in each segment of the body except for the first three and the last. These tubules absorb liquid wastes which are then moved along them by cilia until the external pore is reached. Nephridiopore—this is the external openings from each nephridium and is in the same segment as the coiled nephridium. Nephrostome—the opening from the body cavity that leads into the coiled nephridium. Note that this opening from the body cavity is always located in the segment in front of the coiled nephridium.

Show one nephridium in your drawing.

Reproductive System.

The earthworm is hermaphroditic, that is, each individual has both male and female sex organs. Several of the reproductive organs are too small to be seen without the use of a dissecting microscope. Use a chart, model, or diagram to complete the study of these parts. The following structures should be seen in the dissected specimens; the ovaries and testes may be difficult to find.

Examine the following male organs: Seminal vesicles—three pairs of large white structures extending on the ventral side from segments 9 to 13. The first pair is small and located in segment 9. The next pair is larger and located in segment 11 while the largest pair is located in segments 12 and 13. Seminal receptacles—two pairs of small, round, white structures lying against the ventral wall of segments 9 and 10. Note that the wall is firm and tough. Testes—two pairs of tiny structures enclosed by the vesicles and located in segments 10 and 11. The sperm duct from the testis in segment 11 combines with the other duct from the testis in segment 10 to form a common duct that opens to the outside through a pore in segment 15.

Examine the following female organs: ovaries—one pair of tiny organs on the ventral side of segment 13. The oviducts carry eggs through an opening in segment 14. Clitellum—the coloured band around the outside beginning at segment 30. It produces the tubular cocoon or egg case.

Add the appropriate portions of the reproductive structures to your drawings. Label fully.

Nervous System.

Like that of many other invertebrates, it is made up of a long chain of ganglia located on the ventral side of the body below the digestive tract. In each segment there is one ganglion which has both sensory and motor nerve connections to organs in that segment.

Remove the muscle tissue above the pharynx in segment 3 to expose the cerebral ganglion or brain which is a white, two-lobed structure. Trace the connections from this to the ventral nerve cord and note that this runs the whole length of the body.

Add these structures to your drawings and label fully.

The sense organs of the earthworm consist of separate sense cells. It will respond to touch, light, heat, chemicals, and vibrations.

Cross section of earthworm—posterior to clitellum.

Observe the prepared slide under low power and note the following: cuticule, epidermis, circular muscles, longitudinal muscles, peritoneum, coelom, intestine, typhlosole, enteron, blood vessels, nerve cord, setae (if present).

Draw the cross section and label the above parts.

(6) What is the function of the typhlosole?

(7) What is the function of the cuticle?

LAB. 29

GRASSHOPPER—EXTERNAL FEATURES

Object—to study the general body structure of an insect and its life cycle.

Materials—preserved lubber grasshopper, dissecting needle, hand lens.

Procedure—Notice that the body of the grasshopper consists of three regions: the head, the thorax, and the abdomen.

(a) Head. Observe one pair of antennae, one pair of compound eyes, three ocelli or simple eyes, the upper lip or labrum, one pair of mandibles, one pair of maxillae with maxillary palps, and a lower lip or labium with labial palps.

(b) Thorax. The thorax is made up of three segments: the prothorax, the mesothorax, and the metathorax. Each segment bears a pair of legs. The mesothorax and the metathorax each bear a pair of wings. Notice the manner in which the wings fold. Also notice the veins. Each leg consists of a coxa and trochanter, a long femur, a long spurred tibia, and a tarsus.

(c) Abdomen. The abdomen is made up of eleven segments, but the plates of some of the posterior ones are partially fused. At the end of the abdomen is the external reproductive organ, in the female called the ovipositor. It consists of four prongs.

(1) What is the function of the ovipositor?

Locate the tympanic membrane or eardrum in the first segment, partly covered by the wings. Along the mid-line on each side are the spiracles. Examine them with the hand lens.

(2) What is the function of the spiracles?

Life Cycle of Insects:

Most orders of insects undergo structural changes in the course of their development from the egg to the adult. Depending upon the extent of these changes, the cycle is known as either complete or incomplete metamorphosis.

Make a drawing of a grasshopper from the lateral aspect and label all of the external features referred to in this exercise.

LAB. 30

FISH

Object—to study the external structure of a fish and the use of the various fins.

Materials—preserved fish (perch is satisfactory), hand lens.

Procedure—Observe the following structures: mouth, nostrils, eye, operculum, pectoral fins, pelvic fins, dorsal fins, anal fin, caudal fin, scales, lateral line.

Make a drawing of the fish you are studying and label the above structures.

(1) Does the fish you are studying have teeth? If so, how are they adapted for capturing prey?

(2) What is the function of the nostrils in fish such as the perch?

(3) What is the function of the lateral line?

(4) In the perch, how do the anterior and posterior dorsal fins differ? The fins of a fish are perfectly suited to life in the water. Some serve for locomotion, others as keels to keep the fish on its course, while still others balance the fish while resting, raise it or lower it in the water, or protect it from enemies. Watch the action of a living fish in the water and see if you can determine the use or uses of each of the fins. Record your findings in the form of a chart.

LAB. 31

THE FROG—EXTERNAL FEATURES AND MOUTH

Object—A. to study the external features of a frog and evaluate these features in terms of adaptation to the environment. B. To study the internal features of a frog's mouth.

Materials—Frog—live or preserved, double injected, dissecting tray, dissecting scissors.

Procedure—A. External Features. Place the frog in the tray dorsal side up. Observe: head, external nares (nostrils), eyes, eyelids, tympanic membrane. Notice the skin and the coloration. Also observe the front and hind legs.

(1) Of what adaptive advantage are:

The location of the external nares, the "periscope" eyes, the nictitating membrane, the skin coloration?

Examine the legs.

(2) How many toes are on the front legs? How many on the hind legs?

(3) How is the frog adapted for swimming?

(4) How is the frog adapted for moving on land?

Draw a hind leg and label the following structures: femur, tibio-fibula, tarsals, metatarsals, phalanges.

B. Mouth—Internal Features. Cut the muscles at the hinges of the jaw and open the mouth wide. On the upper jaw notice the lip, maxillary teeth, vomerine teeth, internal nares with a flap of flesh which acts as a valve, eye sockets, openings to the eustachian tubes. At the back of the mouth notice the gullet opening and the glottis. The tongue is attached at the front of the lower jaw. In the male frog only, there are openings to the vocal sacs.

Draw the mouth cavity as seen with the mouth wide open. Carefully label all of the structures mentioned above.

LAB. 32

FROG—DIGESTIVE SYSTEM

Object—To expose and study the digestive tract of a frog.

Materials—frog from previous exercise, dissecting equipment.

Procedure—*Note:* **Before proceeding with a dissection carefully read the instructions. Do not cut or remove any part unless specifically told to do so.**

Place the specimen on its back in the tray and pin it down with a pin through each hind foot.

Lift the skin on the abdomen with the forceps and beginning between the hind legs cut the skin with the scissors along the mid-line to the tip of the jaw. Observe the lymph cavity between the skin and the muscle. Make a transverse cut in the skin on each side between the front and hind legs and pin the flaps back. Notice the large number of blood vessels in the skin. Much of the aeration of the blood takes place in the skin.

Lift the body wall with the forceps and make a longitudinal cut with the scissors, a little to one side of the mid-line, from the hind legs through the shoulder girdle. Pin down the front legs. Note the abdominal vein attached to the inside of the muscles along the mid-line. Insert a dissecting needle between it and the muscle and free it from the muscle throughout its entire length. Do not break it. Now make a transverse cut in the wall at each side as you did with the skin and pin the corners back.

Most of the digestive system is now exposed to view. If your specimen is a mature female taken in the spring the body cavity will be filled with ovaries distended with speckled masses of eggs which crowd all the other organs forward. These eggs will have to be removed before you can proceed.

Observe:

(a) Liver, a large reddish brown organ made up of three lobes at the anterior end of the cavity.

(b) Stomach, usually under the left lobe of the liver. Carefully lift the liver to see it.

(c) Duodenum, the first part of the small intestine curving forward from the stomach.

(d) Pancreas, a thin, irregular shaped, white or yellowish body between the stomach and the duodenum.

(e) Gall Bladder, a small greenish sac between the lobes of the liver. You will have to lift the right lobe of the liver to see it. The bile duct connects the gall bladder to the liver and the duodenum.

(f) Ileum, the main part of the small intestine from the duodenum to the rectum. It is coiled back and forth in the cavity.

(g) Rectum, the large intestine, from the posterior end of the ileum to the cloaca.

(h) Cloaca, the extension of the rectum to the anus. It receives posteriorly the ducts from the kidneys, and anteriorly the urinary bladder.

(i) Mesentery, the thin membrane which suspends the entire alimentary canal from the dorsal wall.

The tip of the heart can be seen between the lobes of the liver. Most of it is probably under the pectoral (shoulder) girdle.

Draw the digestive system, the urinary bladder, the heart and the mesentery as you see them in this view. Make your lines bold and clear. Label fully.

LAB. 33

FROG—HEART AND VASCULAR SYSTEM

Object—to study the circulatory system of a frog.

Materials—frog from previous exercise, dissecting equipment.

Procedure—The Heart and Proximal Blood Vessels. The heart is enclosed in a thin membraneous sac, the pericardium. Remove the pericardium with a needle and make out the light coloured, triangular-shaped, muscular ventricle, and anterior to it the dark brown, thin-walled right and left auricles (also known as atria). Lift the tip of the ventricle and tilt it forward. Note the large post caval vein that passes through the liver, where it is joined by two hepatic veins. It is enlarged above the heart to form the thin-walled sinus venosus. Right and left precaval veins bringing blood from the head and shoulder regions join the sinus venosus from the front. Blood from the sinus venosus empties into the right auricle. Small pulmonary veins from the lungs empty into the left auricle. They are difficult to see.

On the ventral surface observe the large artery that arises from the anterior right corner of the ventricle and passes forward over the right auricle. This is the conus arteriosus. It divides into a right and a left truncus arteriosus. With needle and forceps carefully remove fat and other tissue that covers the trunci. Note that each truncus divides into three arteries: (1) the carotid, which goes forward to the head region; (2) the systemic, the middle one, which goes towards the foreleg, then back under the digestive organs to join the one from the other side to form the dorsal aorta. Lift the digestive organs to one side to see it; (3) the pulmocutaneous which carries blood to the lungs and the skin.

From your observations of the heart and blood vessels, make carefully labelled drawings of the frog's heart and major blood vessels from the dorsal and ventral views.

Follow the dorsal aorta back to where it divides into right and left iliac arteries which go into the hind legs. These are best seen after the digestive tract is removed.

Note several renal arteries that branch off from the dorsal aorta into each kidney, and a large coeliaco-mesenteric artery that branches off to the digestive organs near where the two systemic arches join.

Remove the skin from the right hind leg and spread the bands of muscle tissue along the outer edge of the thigh to expose the femoral vein between muscle tissue above the thigh bone (femur). Follow this forward to where it forks. One branch, the renal portal vein goes to the kidney; the other branch is the right pelvic vein which combines with the left pelvic vein to form the abdominal vein. Follow the abdominal vein which was loosened from the body wall, forward to where it enters the liver.

Move the digestive organs from the frog's right side to the left. Note the two flat, reddish-brown kidneys lying against the backbone. Five pairs of renal veins come from the kidneys and combine to form the post caval vein that takes blood from the kidneys to the heart and enters the sinus venosus on the dorsal side of the heart.

Remove the heart and cut it horizontally with the scissors. Wash it out and study its structure.

(1) Why is the wall of the ventricle so muscular?
(2) Which direction does the blood move through an artery?
(3) Which direction does the blood move through a vein?
(4) Do arteries always carry arterial blood? Explain.

Make a drawing of the circulatory system of a frog as seen in ventral view. Include only the heart and those blood vessels mentioned in this exercise. Shade in the venous system to distinguish it from the arterial.

LAB. 34

FROG—URINOGENITAL SYSTEM

Object—to study the structure of the urinogenital system of a frog.

Materials—frog from previous exercise, dissecting equipment.

Procedure—Cut through the digestive tract at the point where the ileum enters the rectum. Snip free all membranes and blood vessels and remove all of the visceral organs except the posterior portion of the digestive tract, the urinogenitalia, and the blood vessels leading to and from the kidneys.

Observe the following structures:

(a) The kidneys, a pair of large, flattened brownish organs close to the dorsal wall in the posterior part of the body cavity.

(b) The ureters, fine whitish ducts running from the outer border of each kidney to the dorsal wall of the cloaca.

(c) The urinary bladder, a large bi-lobed sac at the posterior end of the body cavity springing from the ventral wall of the cloaca. In most higher animals the ureters connect directly with the bladder.

(d) The adrenal bodies, irregular yellowish lines on the ventral surface of each kidney.

(e) Fat bodies, yellow, branching masses at the anterior end of each kidney.

(f) The testes, in the male, two yellow ovoid bodies lying against the ventral surface of the kidneys. There are very fine tubes leading from them into the kidney through which the sperm pass into the ureters and down to the cloaca.

The ovaries, in the female. If your specimen is a mature female these were distended with eggs and were removed earlier. Otherwise they appear as a pair of dark grey folded bodies lying against the ventral wall of the kidneys.

(g) The oviducts, large, white, thick-walled tubes lying against the dorsal body wall in the female. The anterior end opens into the body cavity near the heart and the posterior end opens into the dorsal side of the cloaca. In the male there are vestigial oviducts which are small and non-functional. The eggs escape from the ruptured ovaries into the body cavity, then pass down the oviducts to the cloaca. They are fertilized after they pass from the body.

Draw the urinogenital system of your specimen, male or female. Label fully.

(1) What is the function of the kidneys?

LAB. 35

FROG—NERVOUS SYSTEM

Object—to study the structure of the brain and spinal cord of a frog.

Materials—frog from previous exercise, dissecting equipment.

Procedure—Remove the skin along the back and top of the skull, then bend the head down slightly. Cut the membranes forming a dark line where the skull joins the vertebra. Then insert sharp pointed scissors in the opening made into the spinal cord and cut the bone of the skull. Break away the bone to expose the brain. Repeat the process to expose the spinal cord.

Notice the following structures: olfactory lobes and associated nerves, cerebrum, optic lobes and optic nerves, cerebellum, medulla oblongata, spinal cord, cranial nerves, spinal nerves.

Remove the brain and spinal cord and make a labelled drawing of the above as seen in dorsal view.

LAB. 36

HUMAN PHYSIOLOGY

Object—to study the circulation of the blood.

Materials—stopwatch, ball point, graph paper, forceps or spatula, stethoscope if available.

Procedure—Select a student in good health, but not fat. Measure his rate of heart beat while seated at rest, using the wrist pressure point (at the base of the thumb on the inner side of the wrist) or a stethoscope if available. Have the student indulge in any convenient strenuous exercise such as runnning 100 yards, running up several flights of stairs, or doing 20 push-ups. Immediately measure the heart rate again and repeat this at 2-minute intervals until it approximates the original value. Make each count for 30 seconds and multiply by two to get beats per minute. Record all times and counts and plot them against each other on squared paper showing also on this the time occupied in exercise.

Note the rapid response of the heart rate to exercise and the slow return to normal. Note also that the respiratory rate increases in response to exercise. If another observer is available this can also be recorded at intervals and plotted.

(1) Do you find a correlation between the responses of respiratory rate and heartbeat to exercise? Explain.

(2) Assuming that the heart pumps 70 cc. of blood per contraction and that the experimental animal has a blood volume of 5 litres, calculate the maximum and minimum times for the entire blood volume to pass through the heart in your experiment.

Using a student with similar qualifications, have him distend the veins of one hand and arm by swinging it around rapidly three or four times. Then have him lie down on his back on the bench with this arm hanging down over the edge. Use the smoothly rounded handle of a pair of forceps, or a small spatula to move the blood distally in each visible vein in turn. Watch for the appearance of small swellings in the vein distal to the instrument, indicating the location of valves. Confirm each location by noting that when the instrument is moved distally from it, the vein between it and the instrument is emptied of blood and "disappears". Mark all valves found with a cross line with a ball point and an arrow indicating the direction of flow. The back of the hand and the inner side of the forearm are the best areas to work on. Make a drawing of at least one of these areas, indicating the location of valves on it. If time permits make a similar study of a leg below the knee.

(1) Which way does the blood flow in the veins?

(2) How many valves did you locate and what was the smallest vein in which you found a valve?

(3) What does the "disappearance" of a vein when it is emptied of blood mean in terms of the source of the blue color of veins? Give two reasons for the absence of blue color from arteries.

(4) Do you find valves more closely spaced in the veins of the arm or of the leg? Why?

This experiment was first done by William Harvey about 1615.

LAB. 37

HEREDITY—POPULATION GENETICS

Object—to study inherited traits in a class of sudents.

Materials—phenylthiocarbamide (PTC) test paper — 4 pieces, sodium benzoate taste test papers—4 pieces, color blindness test cards—1 set.

Procedure—Taste discrimination: PTC test paper: Each student should chew a strip of PTC test paper. Record the results as sweet, sour, salty, bitter, or no taste.

Sodium benzoate test papers: Repeat as with PTC test papers.

The instructor will consolidate on the blackboard the results for the entire class. Work out and record the percentage of the different types of "tasters" and "non-tasters" in the class.

(1) Based on the results of this experiment, is the trait for tasting dominant or recessive? Explain.

Color blindness—Work in pairs—one partner acting as the subject, the other as the examiner and recorder. When one partner has been tested, exchange roles and test

the other partner. Instructions for the use of the test cards will be outlined by your instructor who will also tabulate your results.

(2) Color blindness is said to be a sex-linked character. Explain.

(3) Is your answer to question two substantiated by the results obtained on this experiment? If not, offer any possible explanations.

Ear lobes—free or attached.

Observe the ear lobes of members of your group to determine whether they are free (not attached at the lower end) or attached. As above, a consolidated tabular record should be prepared and recorded.

(4) After observing this trait in your parents and siblings, can you determine if this is a dominant or recessive trait? Explain your answer.

Eye color—Prepare a tabular record showing the distribution of eye color for members of your class. Follow the procedure previously used.

(5) Which eye color appears to be dominant and which recessive? Explain.

(6) Explain why albinos have pink eyes.

(7) Does there appear to be any correlation between eye color and need for eye glasses? If so, what correlation?

Appendix A

1. FIELDWORK, CULTURES, REAGENTS AND APPARATUS

Introduction—What you learn about plants and animals in the laboratory becomes meaningful when you see them in their natural habitats. Only when field work becomes a part of education will man come to understand what overpopulation will do to his environment, and science come to face the problem of a replacement for the Malthusian checks it has so proudly removed from our own increase in numbers. To make the most of your opportunities for field work you must prepare for them ahead of time in your reading and laboratory work and by planning and assembling the materials you will need (see below). You must also follow up your field trips, firstly by transcribing your observations into permanent records — such records accumulating in a school for a number of years can become important scientific contributions. Secondly by labelling, preserving, and where possible, identifying the specimens you bring back. In this way school collections of plants and insects; shells, skins and skeletons; fossils and fish, flowers and fungi, can be built up for future students.

2. FIELD EQUIPMENT & PROCEDURE & CULTURE METHODS

The following minimum field equipment will be needed: collecting syringe*, dip nets (at least one of fine mesh), field notebook and pencil, field thermometer, forceps, haversack, insect net and killing bottles, knife, large and small bottles, measuring tape, newspaper, plastic bags, rubber boots, trowel, wax marking pencil

* Two collecting syringes may be made by heating the centre of a 2 to 3 foot length of about ¼ inch glass tubing and drawing it out to make a constriction which is then cut to give two medicine dropper ends. Fit rubber bulbs to the other ends.

Several of the laboratory exercises which are to be done in this course, will require samples of organisms to be found in most ponds or sloughs. Before making your collection, set up several battery jars or small aquaria in a well lighted spot in the classroom (preferably not in direct sunlight). These jars will be used to receive the samples collected and one should plan on maintaining the cultures for several months. Each jar should be provided with a glass cover to retard water loss by evaporation. It is also desirable to have one or more jars fitted with aeration equipment in order that animal forms such as various insect nymphs will not die from oxygen deficiency. Collect sufficient water from the pond to initially fill all receiving jars. Subsequent water loss may be replaced with distilled or clean chlorine-free water from any source.

It is beyond the scope of this appendix to itemize in detail the organisms to be sought. In general, such things as algae and larger aquatic plants and all forms of aquatic animal life should be collected in as great a diversity as the environment allows—do not overlook organisms in the surrounding mud and in animal tracks filled with water. The larger aquatic plants can be wrapped in wet newspaper for transport back to the laboratory. Take samples from various strata of the pond including the bottom (the collecting syringe is valuable for collecting from the material settled on the bottom). Use dip nets to collect swimming forms and don't overlook aquatic insects such as beetles, water boatmen, back swimmers, and aquatic nymphs. Use the field notebook to record such data as: date of collection, location of pond, stratum of pond where a particular sample was collected, water temperature, weather, activity, and so on.

For one sample particularly suitable for Lab. 14 collect one large jar of water, dead grass and organic debris from the pond. Set this jar in a moderately dark and warm location for a week or more. When a scum has formed on the surface (and obvious decay is taking place!) the scum will contain vast numbers of protozoa and rotifiers.

Desmids (Lab 8)—Desmids are unicellular algae belonging to three families of the Order Zygnematales of the phylum Chlorophyta. The Family Desmidiaceae is made up of members which typically contain one nucleus and few relatively large, ornate chloroplasts. Large pyrenoids are generally present. Many forms appear as two mirror-image semi-cells joined at a constricted isthmus. In these forms, a vacuole is often present at each end of the cell and vibrating gypsum granules are usually to be seen in the vacuolar contents. The desmids are among the most beautiful of the plants. Their intricate shapes and decorations provide a memorable sight for even the casual microscopist.

Desmids generally favor a soft water environment where the water is slightly acid. Such conditions are usually found in acid bogs such as *Sphagnum* bogs and typical northland muskeg. Most soft water sloughs (not alkaline) with considerable decaying vegetation in them will contain numbers of desmids. Desmids may frequently be found in the marginal waters of such acid ponds and may be collected by dipping up the water. They may also be collected by straining them from the water with a fine mesh dip net or merely by collecting masses of filamentous algae amongst which the desmids will be found (Refer to Ward's Culture Leaflets for culture methods for desmids).

Filamentous Green Algae (Lab. 9)—There are many genera of algae of this general description but not all are suitable for our purposes. Those genera best suited belong in the Order Zygnematales. With the exception of the desmid families, plants in this order are unbranched filaments without basal-distal differentiation. The cells of the vegetative filaments are long or short cylinders. Each cell is bounded by a distinct cell wall and in most forms a slimy mucilaginous sheath covers the filament. The slimy feeling due to this sheath is a valuable indicator when seeking specimens in the field.

One of the most characteristic features of this order is the relatively large size of the chloroplasts, usually one or two in each cell. In *Spirogyra* however, there may be from one to sixteen spiral chloroplasts, depending upon the species. Pyrenoids in the chloroplasts are large and conspicuous.

Sexual reproduction is typically by conjugation. Either all or part of the vegetative cell content serves as a gamete, union being achieved by one or both gametes passing through a tube which forms between the cells of two filaments. Reproductive material often can be identified in the field, especially in *Spirogyra, Mougeotia,* and *Zygnema,* by the foamy, brownish or dirty appearance of the plant masses.

While *Spirogyra* has, apparently by tradition or habit, been the subject of study at this level in the past, it really is no better than either of its close relatives *Mougeotia* or *Zygnema.* (See Modern Biology, Fig 9—6, page 99.) All three genera are widely distributed in this province and there is really no excuse for not having living specimens for study—especially during fall and spring. Masses of these bright green plants are often found floating on the surface of ponds and sloughs. The mats appear foamy during sunny weather due to the oxygen bubbles liberated by photosynthesis.

There are several suggested procedures for culturing these algae. Putting them in a large clear glass jar along with the water in which they were growing in the field is one method. The jar must be kept in a well lighted location, but avoid direct sunlight as it causes the water to get too warm. Samples can be kept in a refrigerator for 2 or 3 weeks.

The following culture method has been found to work very well for many types of algae and is included for your information:

Place about a gram of powdered calcium carbonate in a 250 cc. Erlenmeyer flask and cover with ¼-inch of soil (avoid clay and soil which might be contaminated with herbicides, fertilizers, insecticides or fungicides). Add 150 cc. of distilled water. Plug with cotton. Sterilize this at 100 degrees C., without pressure, for 1 hour on each of three consecutive days. Add several filaments of the alga which you wish to culture and store in an area which is strongly lighted. During the short days of winter it may be necessary to provide some artificial lighting.

Paramecium **and** *Euglena* (Labs. 12 and 14)—Various ciliate Protozoa including species of *Paramecium* may be obtained in large numbers from a hay infusion. Some hay or straw is added to a jar of water which is then kept out of sunlight for a few days. Such a preparation may also be sterilized and then inoculated with any species, which will usually multiply rapidly. Bran, cracked wheat, horse or cow manure and various other organic materials also make satisfactory infusions in water for various protozoa. Many such preparations, if they are kept in sunlight, end up as cultures of *Euglena.*

Rhizopus **Culture:**

Culture medium for bread mold.

Glucose—20 grams, rolled oats—10 grams, powdered agar—17 grams, distilled water—1000 ml., salt (NaCl)—1 small pinch.

Mix the above ingredients. Add enough vinegar to adjust the pH to about 5.5. Heat to boiling and cook until all agar has dissolved. Pour into clean petri dishes (half fill each dish) and allow to stand until cool. DO NOT put the covers on the dishes until cooled. Save about 20 ml. of the medium in a beaker for use in preparing slides. Cover the dishes and autoclave at 15 p.s.i. for 20 minutes. Cool and store inverted plates in a refrigerator until needed.

In addition to the above medium, several pieces of moldy bread will be required for "seeding" the slides. There is usually no problem in getting moldy bread!

When the students are preparing their culture slides, keep the beaker of culture medium sufficiently warm (melted) so that they will be able to get 2 or 3 drops with a dropper to place on their slides.

Amoeba **Culture** (Lab. 13):

A. *proteus* and other species may most readily be collected at the interface between water and organic sediment in pools or ponds of rather clean water. Samples of this interfacial material should be taken into the laboratory in water and allowed to settle in tall jars. Specimens can usually be collected from the top of the sediment in these jars. *Amoeba* will multiply in glass bowls containing a layer of clean very fine sand and distilled water with 0.1 gm. NaCl, 0.004 gm. KC1, 0.006 gm. $CaCl_2$, and 20 grains of polished rice per liter. Cultures should be covered and kept in the dark, or at least away from sunlight, and at a temperature between 70 and 75 degrees F. *Amoeba* may continue to multiply for several months without attention other than the addition of water and occasionally a grain or two of rice. The sand also serves to support cover glasses above the specimens when samples of the culture are being examined.

Mosses and Liverworts (Lab. 15):

Plants of either or both of these groups are available in most areas of the province and should be collected in the early fall. Both keep well for several months in a terrarium. (A two-quart jar is suitable as a terrarium.) Keep the plants moist and store in a cool light location but not in direct sunlight.

In case you are not familiar with liverworts, they may usually be found in wet, deeply-shaded marshy areas. They may often be found in crevices along well shaded streams or permanent ponds. Collect mature specimens. Both male and female plants should be collected so that you can show both the archegonial and antheridial structures (see text, pp. 129-130).

Ferns (Lab. 16):

One can usually find ferns in the same environment as mosses and liverworts. Enough specimens should be collected for class use. Also, collect fronds with mature sori so that spores may be cultured. See pages 394-387 in Morholt (Appendix A, 4).

If wild ferns are not available, any cultivated potted variety would be acceptable but one should be prepared to expose the rhizomes and roots in order that students may see the gross structure of the plant.

Grass Seedlings (Lab. 17):

Float 50-100 grass seeds on the surface of a container of water. They will germinate and be ready for use in about 6 days.

Corn and Bean Seedlings (Lab. 25):

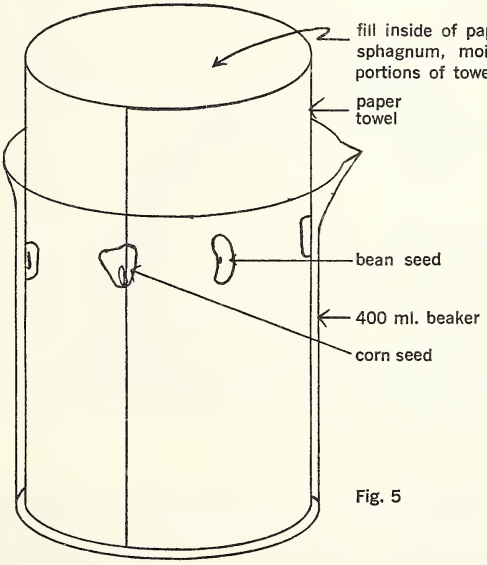


Fig. 5

Prepare an adequate number of growing devices by setting them up as shown in this sketch. Students will be able to watch root development from day to day. It requires about 7-8 days for the seedlings to grow 2 inches tall.

Hydra **Cultures** (Lab. 26):

It is suggested that a culture of living *Hydra* be maintained in the laboratory at least during the period of time when students are working with these animals. If the animals are not collected in the field they may be ordered from a biological supply house.

The collection and culturing of *Hydra* is discussed on pages 345-346 of Morholt (Appendix A, 4). One of the biggest problems in culturing *Hydra* is maintaining a food supply. *Daphnia* are most suitable and may be found in sloughs during the summer. They are easily collected with a fine mesh dip net. However, brine shrimp may be used as a substitute. Brine-shrimp eggs keep in a dried condition for a year or more. When they are sprinkled on the surface of a salt solution, they hatch in 48 to 60 hours. The eggs are usually available in aquarium and pet stores.

Two or three days before starting the *Hydra* populations, set up a culture of brine shrimp as follows: Add about 5 g. of sodium chloride to 500 ml. of tap water and stir to dissolve. Pour enough of this solution into two brine-shrimp culture dishes to bring the level of the solution within about 3 cm. of the rim. Any remaining solution can be set aside for the next day. Place the culture dishes where they will be at room temperature. Sprinkle dried brine-shrimp eggs on the surface of the solution. They should not be so thickly strewn that they touch each other. **Do not move the dishes after the eggs are in them.** Two new dishes of the brine-shrimp culture should be set up each day until two days before the end of the experiment. Each day, after the shrimp have been removed, the same dishes can be used to set up the new cultures.

To remove the brine-shrimp from the culture dish arrange an apparatus as shown in the sketch.

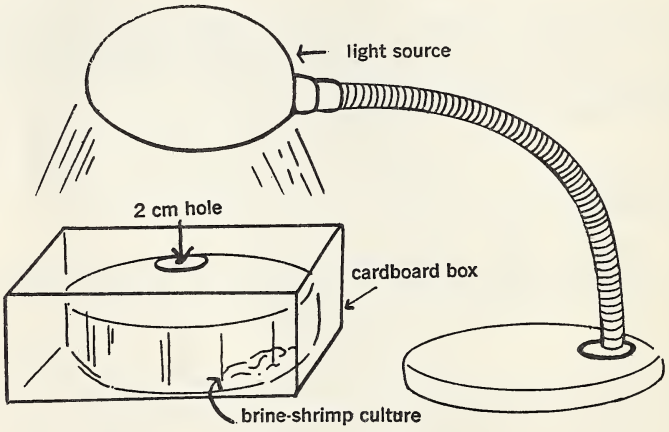


Figure 6.

Allow the light to shine through the hole in the box for about 10 minutes. During the 10 minutes, arrange another apparatus by draping a strainer (a piece of nylon fabric works best) over a beaker. Use a bulb syringe to suck up the brine shrimp from the culture dish. Strain and rinse with fresh water (not chlorinated) by gently squirting fresh water over the strainer holding the shrimp. Rinse the shrimp off the nylon strainer into the *Hydra* culture.

Planarians (Lab. 27):

A good outline for the culture of living planaria is given on pages 346-347 of Morholt (Appendix A, 4).

Students may be encouraged to do some further investigations using planarians to demonstrate regeneration or as subjects for the study of behavior.

3. KEYS AND IDENTIFICATION

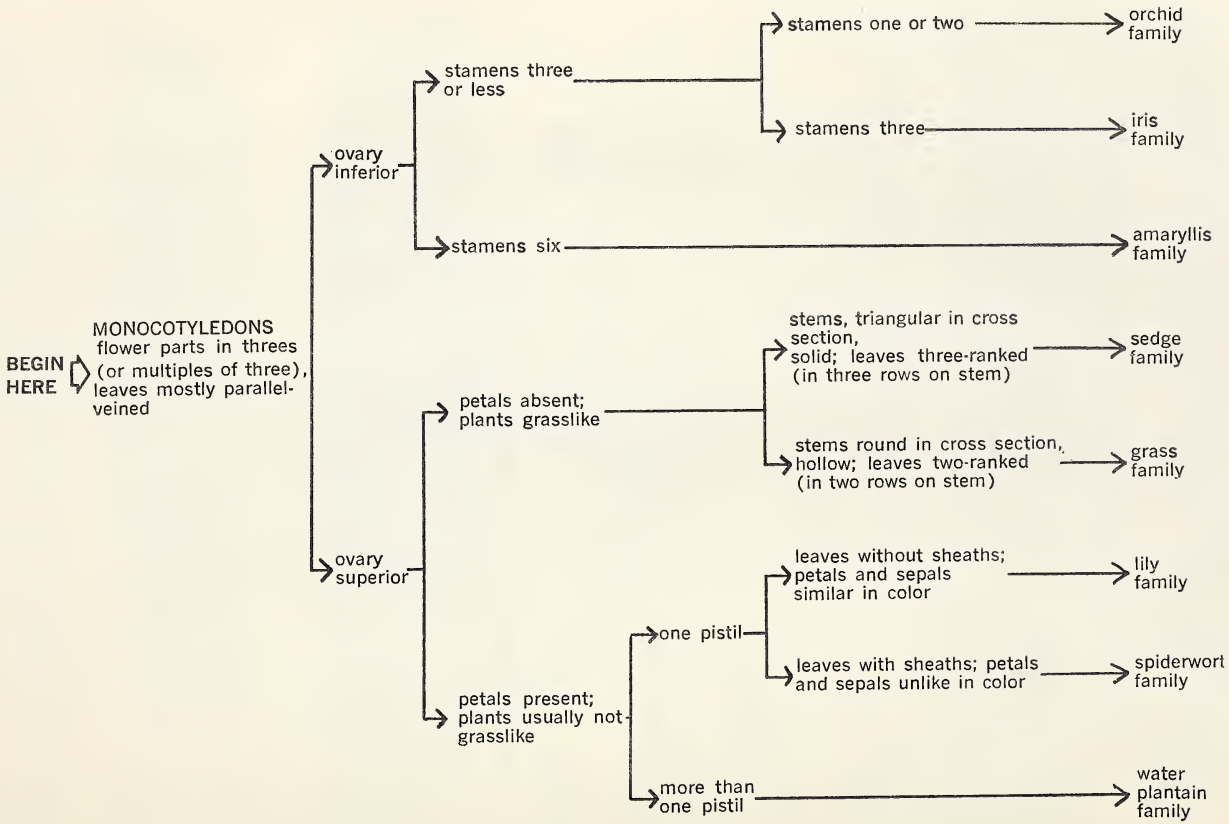
The following publications (Appendix A, 4, p. 56 ff) will be found useful in attempting identifications, but with many organisms you must not expect, as yet, to be able to name them as to species. They will also help you in preserving and looking after specimens.

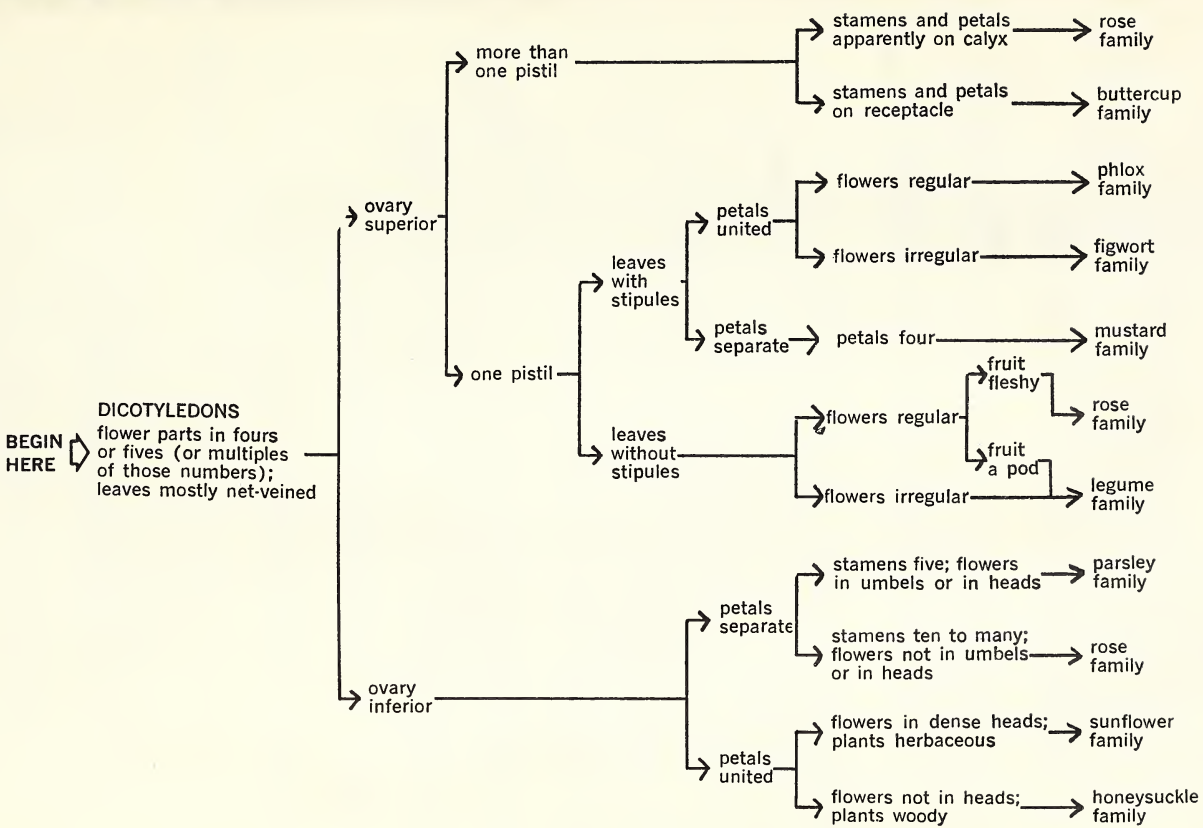
Each student should have, for his own use after a field expedition, a copy of one or other of the items in column 2 so that each may come in contact with some of the problems of identification and get some experience in the use of keys, while the class as a whole can cover most of the groups of plants and animals likely to be encountered. Collecting is a waste of time unless every specimen you collect is labelled with your name, and the date and precise location of collection and unless the observations you make on it are recorded in a field log book or similar permanent record for the class.

Group	Field Guides and Keys	Reference Works
Algae	19, 22	10 & 11
Higher Plants	3 and this manual pp. 52 & 53	17, 10
Trees	8	
Aquatic Plants and Invertebrates	15, 18, 26	20
Insects	14, 13	9, 12
Vertebrates	this manual p. 54	27, 24
Fish, Amphibians and Reptiles	7, 15, 5, 6 and this manual pp. 54 & 55	28, 29
Birds	23, 21	2, 25
Mammals	24, 4	1

Key to Some Important Families of Flowering Plants

Beginning at the large arrow, you will find two alternative paths, requiring, for example, a choice between "ovary superior" and "ovary inferior". Select the correct one for the specimen you are studying. Follow the path from this selection to the next fork in the path and again choose the route indicated by the characteristics of the specimen. Continue in this way until you reach the end of the route, where you will find the name of a family. Check this answer by comparing your specimen with a description of the family in a text or reference book.


BEGIN HERE
MONOCOTYLEDONS
flower parts in threes (or multiples of three), leaves mostly parallel-veined
ovary inferior
stamens three or less
stamens one or two
orchid family
stamens three
iris family
stamens six
amaryllis family
ovary superior
petals absent; plants grasslike
stems, triangular in cross section, solid; leaves three-ranked (in three rows on stem)
sedge family
stems round in cross section, hollow; leaves two-ranked (in two rows on stem)
grass family
petals present; plants usually not grasslike
one pistil
leaves without sheaths; petals and sepals similar in color
lily family
leaves with sheaths; petals and sepals unlike in color
spiderwort family
more than one pistil
water plantain family


BEGIN HERE
DICOTYLEDONS
flower parts in fours or fives (or multiples of those numbers); leaves mostly net-veined
ovary superior
more than one pistil
stamens and petals apparently on calyx
rose family
stamens and petals on receptacle
buttercup family
one pistil
leaves with stipules
petals united
flowers regular
phlox family
flowers irregular
figwort family
petals separate
petals four
mustard family
leaves without stipules
flowers regular
fruit fleshy
rose family
fruit a pod
flowers irregular
legume family
ovary inferior
petals separate
stamens five; flowers in umbels or in heads
parsley family
stamens ten to many; flowers not in umbels or in heads
rose family
petals united
flowers in dense heads; plants herbaceous
sunflower family
flowers not in heads; plants woody
honeysuckle family

Dichotomous Key to Selected Invertebrate Phyla

1a. Body symmetry radial ____ go to 2
1b. Body symmetry not radial ____ go to 3

2a. Tentacles present, body soft ____ Phylum Coelenterata
2b. Tentacles absent, body hard and rough ____ Phylum Echinodermata

3a. Exoskeleton present ____ go to 4
3b. Exoskeleton absent ____ go to 5

4a. Jointed legs present ____ Phylum Arthropoda
4b. Jointed legs absent ____ Phylum Mollusca

5a. Body segmented ____ Phylum Annelida
5b. Body not segmented ____ Phylum Platyhelminthes

Dichotomous Key to Classes of the Subphylum Vertebrata

1a. Hair present ____ Class Mammalia
1b. Hair absent ____ go to 2

2a. Feathers present ____ Class Aves
2b. Feathers absent ____ go to 3

3a. Paired fins present ____ go to 4
3b. Paired fins absent ____ go to 6

4a. Jaws present ____ go to 5
4b. Jaws absent ____ Class Agnatha

5a. Skeleton bony ____ Class Osteichthyes
5b. Skeleton cartilaginous ____ Class Chondrichthyes

6a. Skin scales present ____ Class Reptilia
6b. Skin scales absent ____ Class Amphibia

KEY TO AMPHIBIA OF ALBERTA

1 (4) Tail present.

2 (3) Irregular greenish-yellow band or series of blotches along middle of back; digits elongate, cylindrical and without horny tips; size to 128 mm.—*Ambystoma macrodactylum*—Long-toed Salamander.

3 (2) No dorsal band on back; body mottled with black on olive or yellow ground color; digits short, broad and depressed, and having horny tips; size to 150 mm. or more—*Ambystoma tigrinum*—Tiger Salamander.

4 (1) Tail absent.

5 (12) Waist wide; body broad and thick; hind limbs short.

6 (7) Pupil of eye vertical; paratoids* absent; skin relatively smooth—*Scaphiopus bombifrons*—Plains Spadefoot Toad.

7 (6) Pupil of eye horizontal or round, paratoids present; skin warty.

8 (11) Cranial crests present.

9 (10) Cranial crests divergent at rear; raised horny boss extending from snout to just in front of eyes; parotoids small, widely separate and extending obliquely downward—*Bufo cognatus*—Great Plains Toad.

10 (9) Cranial crests parallel; boss extending from snout to rear of eyes; parotoids large, less widely separated and not extending obliquely downward—*Bufo woodhousei*—Dakota Toad.

11 (10) Cranial crests absent—*Bufo boreas*—Boreal Toad.

12 (5) Waist narrow; body relatively long and narrow; hind limbs long.

13 (14) Small disks on ends of digits; ventral surface granular in appearance; size small—to 1½ inches in length—*Pseudacris triseriata*—Chorus Frog.

14 (13) No disks on digits; venter smooth; size medium or large.

15 (16) Red color on underparts; back roughened with tubercles; spots on back with light centers—*Rana pretiosa*—Spotted Frog.

16 (15) No red color on underparts; back not roughened with tubercles; no light centers to spots on back.

17 (18) With conspicuous rounded or elongated spots on back; dorsal spots with narrow light border; no dark patch behind eye—*Rana pipiens*—Leopard Frog.

18 (17) Without large spots on back; dorsal spots with no light border; dark patch behind eye with a bar running forward to snout—*Rana sylvatica*—Wood Frog.

*Large, paired, and slightly raised glands behind the eyes.

KEY TO REPTILES OF ALBERTA

1 (2) Body lizard-like and possessing numerous spines and tubercles—*Phrynosoma douglassi*—Short-horned Lizard.

2 (1) Body snake-like.

3 (12) No rattle on end of tail, no pit between eye and nostril.

4 (11) Snout rounded, not protruding.

5 (10) Body bearing 1 dorsal and 2 lateral stripes; scales in 19-21 rows.

6	(7)	Lateral stripes on 3rd and 4th rows of scales—*Thamnophis radix*—Plains Garter Snake.
7	(6)	Lateral stripes on 2nd and 3rd rows of scales.
8	(9)	Scales in 21 rows at anterior end of body; 8 upper lip plates (labials); both pairs of chin shields about equal in size—*T. elegans*—Wandering Garter Snake.
9	(8)	Scales in 19 rows; 7 upper lip plates; rear pair of chin shields considerably longer than front pair—*T. sirtalis*—Red-sided Garter Snake.
10	(5)	Body unstriped; scales in 25 - 35 rows—*Pituophis melanoleucus*—Bull Snake.
11	(4)	Snout upturned with lower surface flat—*Heterodon nasicus*—Plains Hog-nosed Snake.
12	(3)	A rattle on end of tail; deep pit between eye and nostril—*Crotalus viridis*—Prairie Rattlesnake.

Note: In 1962 another species of reptile, the Western Painted Turtle—*Chrysemys picta*—was found in Alberta for the first time, in the Milk River of Southeastern Alberta.

4. REFERENCES

Such books as the following serve as excellent references and must be available for use. They not only give ideas as to what to look for and where to look, but also indicate suitable culture methods. The seventeen items marked with an asterisk (*) are essential for the study and identification of specimens collected in the field. Enough copies should be available in the classroom for each student to have one or other of them.

1. Anderson, R. M., *Catalogue of Canadian Recent Mammals*. Bulletin No. 102, National Museum of Canada, Ottawa. Queen's Printer.
2. Austin, O. L., and A. Singer, *Birds of the World*. Golden Press, New York. 1961. 316 pp.
3. *Budd, A. C., *Wild Plants of the Canadian Prairies*. Dept. of Agriculture, Ottawa. Queen's Printer. $1.50. Keys in this manual.
4. *Burtt, W. H., and R. P. Grossenheider, *A Field Guide to the Mammals*. Houghton Mifflin & Co., Boston. 1952.
5. *Carl, G. C., *The Amphibians of British Columbia. 1959*. British Columbia Provincial Museum, Victoria.
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11. Hill, J. B., Overholts, L. O., and Popp, H. W., *Botany, A Textbook for Colleges*. 2nd Edition. McGraw-Hill, Toronto. 1950. 672 pp.

12. Imms, A. D., *A General Textbook of Entomology*. 9th Edition. Methuen, London.

13. *Klots, A. B., *A Field Guide to Butterflies*. Houghton Mifflin Co., Boston. 1951. 349 pp.

14. *McMullen, R. D., *The Insect Collector's Guide*. Entomological Society of Alberta. 1955, (revised 1964). $0.25.

15. *Morgan, A. H., *Field Book of Ponds and Streams*. 1930. G. P. Putnam's Sons, New York. 448 pp.

16. Morholt, E., Brandwein, P. F., and Joseph, *A Sourcebook for the Biological Sciences*. Harcourt, Brace and Company, New York. 1958.

17. Moss, E. H., *Flora of Alberta*. University of Toronto Press, Toronto. 1960. $6.60. 546 pp. (vascular plants).

18. *Needham, J. G., and P. R. Needham, *A Guide to the Study of Fresh-water Biology*. Comstock Publishing Company, Ithaca, N.Y. 1941. 189 pp.

19. *Palmer, C. Mervin. 1950. *Algae in Water Supplies*. An illustrated manual on the Identification, Significance, and Control of Algae in Water Supplies. U.S. Dept. of Health, Education and Welfare, Public Health Service Publication No. 657. 1959. Supt. of Documents, U.S. Govt. Printing Office, Washington 25, DC. $1.00. viii + 88 pp.

20. Pennak, R. W., *Freshwater Invertebrates of the United States*. The Ronald Press, New York. 1953. 769 pp.

21. *Peterson, R. T., *A Field Guide to Birds*. Houghton Mifflin Company, Boston. 1947. 290 pp.

22. *Prescott, G. W. 1954. *How to Know the Freshwater Algae*. William C. Brown Company, Dubuque, Iowa.

23. *Salt, W. R., and A. L. Wilk, *The Birds of Alberta*. 1958. Hamly Press, Edmonton, Alberta. 511 pp. $5.00.

24. *Soper, J. D., *The Mammals of Alberta*. Hamly Press, Edmonton, Alberta. 1964. $5.00. 404 pp.

25. Taverner, P. A., *Birds of Canada*. 1934. National Museum of Canada, Ottawa. 446 pp.

26. *Ward's Culture Leaflets. Ward's Natural Science Establishment, P.O. Box 1712. Rochester, N.Y.

27. Young, J. Z. 1962. *The Life of Vertebrates*. Oxford University Press, London. 820 pp. 70s. (In U.K.)

28. Zim, H. S., and H. H. Shoemaker, *Fishes*. 1956. A Golden Nature Guide, The Musson Book Co., Ltd., Toronto.

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5. SOLUTIONS, REAGENTS & APPARATUS

Iodine - Potassium Iodide (I-KI) Solution (Labs. 4, 5, 23):

Dissolve 3 grams of potassium iodide in 25 ml. of water. Add 0.6 grams of iodine crystals and stir until dissolved. Make up to 200 ml. with distilled water. Store in a dark bottle.

Gram Staining Procedure (Lab. 6):

Smear a small amount of the sample to be stained on a clean slide. Allow it to dry and then fix it in a Bunsen flame (wave the slide three times through the flame).

Add 3 or 4 drops of crystal violet stain* for one minute. Rinse gently in water. Flood the slide with Gram's iodine (2 grams of KI and 1 gram of iodine crystals in 300 ml. of distilled water) for one minute. Rinse gently in water. Decolorize with alcohol-acetone, a 50-50 mixture of acetone and ethyl alcohol. The decolorizing process is critical. Apply the alcohol-acetone dropwise until most of the violet dye has disappeared. Two or three washes over a period of about 15 seconds will usually give adequate but not excessive decolorization in water.

Now counterstain the slide with safranin** for 30 seconds. Rinse in water and blot dry.

*Crystal violet, ammonium oxalate solution. Mix together 5 ml. of crystal violet stock solution and 5 ml. of 95% ethanol. To this add 40 ml. of a 1% aqueous solution of ammonium oxalate.

**Safranin dye. Add 3.4 grams of powdered dye to 100 ml. of 95% ethanol. Let it stand for 48 hours. Stir frequently; filter and store.

Methylene Blue Stock Solution (Lab. 6, Part II):

Add 1.48 grams of powdered methylene blue dye to 100 cc. of 95% ethyl alcohol. Stir well. Let stand for about 2 days, stirring frequently; filter and store.

Bacteria Culture Medium (Lab. 6, Part III):

A suitable culture medium for bacteria is prepared as follows:
To 1000 cc. of distilled water add the following:
Beef extract—3 grams
Tryptone—5 grams
Glucose—1 gram
Agar (powdered)—15 grams

Bring to a boil and stir so that all ingredients are dissolved. Allow to cool enough for safe handling and pour into the prepared culture dishes or tubes.

Sterilizing Procedure:

Culture media, glassware, swabs, etc. may be sterilized by placing them in an autoclave. (Pressure cookers with pressure gauges may serve as autoclaves but manufacturers' instructions should be checked as to their safety for this purpose.)

Respirometer for Fermentation Demonstration (Lab. 10):

This device consists of two tubes of such a size that the lipless one fits snugly but freely inside the other as shown in the sketch.

The nutrient solution and yeast mixture is placed in the smaller of the two test tubes. (Fill the tube completely.) Lower the larger tube over the small one and, using a pencil, push the smaller tube all the way up into the larger tube (Fig. 7, A.). Invert and incubate (Fig. 7, B.).

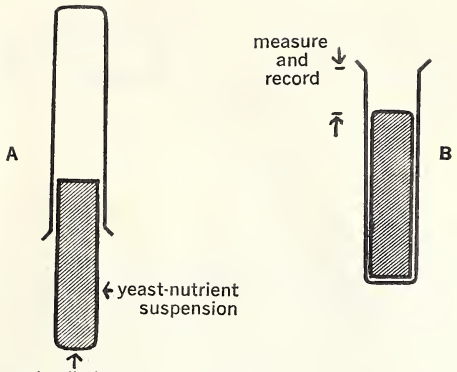


Fig. 7

Dialysis Chamber (Lab. 19):

Material—⅝″ dialysis tubing—4″ per four students; #0 1-hole rubber stopper—1 per four students; string—12″ per four students.

Wet the dialysis tubing and open it by rolling it between the fingers. Hold one end of the tubing and blow on the open end to expand it fully. Wet the rubber stopper and slip the open tubing over the small end of the stopper. Work the stopper all the way into the tubing and with the string bind the tubing tightly to the stopper. Tie a simple knot in the other end of the tubing. See Fig. 9.

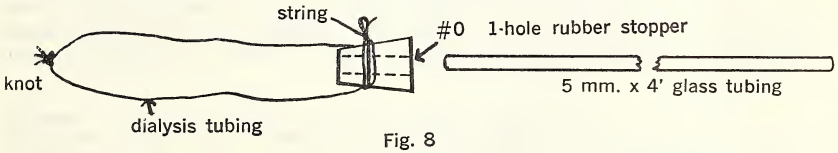


Fig. 8

Enough saturated glucose solution should be prepared to fill the chambers of all groups. In preparing the solution, add enough glucose so that about ¼″ of undissolved crystals remains in the bottom of the container. Students will suck up the saturated solution and the crystals with the rubber bulb syringe to fill their dialysis chambers. Add several drops of methylene blue dye to the solution so that it may be more easily observed in the osmosis apparatus. The teacher should set up one osmosis apparatus with water in the chamber, to act as a control and provide a reference for the class.

After the experiment is completed, have the students rinse out the dialysis chambers in clean water. Store them in a sealer so that they are submerged in a 10% solution of glycerine and water. They may be used repeatedly.

Diffusion Tube (Lab. 19):

Material: glass tube 4′ x 45 mm. o.d., absorbent cotton, phenolphthalein solution, filter paper, 12 inch ruler, wax marking pencil.

With the wax pencil, mark the tube at A, B, C, and D. Moisten pieces of filter paper with phenolphthalein solution and place them inside the tube at the locations marked. Plug both ends of the tube with absorbent cotton. This diffusion experiment should be done before the phenolphthalein solution dries. See Fig. 9.

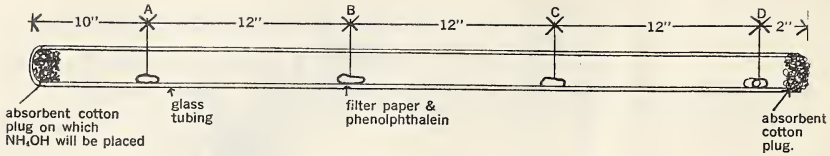


Fig. 9

A Section of a Tree Trunk (Lab. 20):

Prepare at least one hardwood section (deciduous tree, e.g. poplar, willow, balsam), and at least one softwood section (conifer, e.g. spruce, pine). The sections should be of as great a diameter as possible. Use dry sections. A fine-tooth band saw is best for cutting them. Sand the cut surfaces perfectly smooth to bring out the different tissues and finish with several coats of urethane varnish or shellac. (Dilute shellac for the first coat with an equal volume of alcohol.) After the first coat of shellac is applied one can print labels directly on the sections to aid students in identifying the various tissues and regions. See text fig. 16-6 p. 172.

Brom-thymol Blue Stock Solution (Lab. 23):

Dissolve 40 mg. of powdered brom-thymol blue dye in 3 ml. of 95% ethanol. Add this to 96 ml. of distilled water. Brom-thymol blue solution is yellow in an acid (pH 6) and blue in a base (pH 7.6). It is greenish between these two points.

Starch Test (photosynthesis) (Lab. 23):

Boil the leaves for a few minutes in water in a small beaker. Transfer the leaves from the boiling water to a beaker half full of ethyl or methyl alcohol. Warm on an electric hot plate or in a water bath. **Caution: Alcohol is Inflammable.** Chlorophyll is soluble in alcohol, and the pigment should be extracted in about 4-5 minutes.

Rinse the blanched leaves in water, spread them flat in open petri dishes and flood with a dilute aqueous iodine solution. After three or four minutes rinse off the excess iodine solution; hold the leaves up to the light to show the blackish areas which indicate the presence of starch.

MATERIALS AND EQUIPMENT

It is assumed that routine reagents and glassware will be available in the laboratory.

Chemicals

Acetic acid
Acetone
Agar-agar (powdered)
Ammonium oxalate
Beef extract
Benedict's solution
Brom-thymol blue (powder)
Calcium carbonate (powdered)
Carbon tetrachloride
Chloroform
Corn starch
Crystal violet dye
Dextrose (glucose)
Ethyl alcohol (denatured)
Ether (diethyl)
Formaldehyde
Iodine crystals
Methyl alcohol
Isopropyl alcohol
Methylene blue dye
Methyl cellulose
Potassium iodide
Safranin dye 1% aqueous
Tryptone
Yeast

Glassware

Beakers
Bottles, reagent
Cover slips for slides
Flasks, Erlenmeyer
Jars, battery
Medicine droppers
Pipettes
Petri dishes
Rods, stirring
Slides, microscope, plain and 1-cavity
Test tubes (18 x 150 mm.)
Respirometers (See Appendix A)
Tubing, glass misc.
Tubing, glass—45 mm. o.d. x 4′ (Diffusion tube, see Appendix A)
Vials, screw cap

Expendable Supplies

Adhesive tape
Applicator sticks
Brushes, test tube
Bulbs, rubber (30 ml.)
Absorbent cotton
Nonabsorbent cotton
Dialysis tubing (⅝″ diameter) (See Appendix A)
Files—triangular
Filter paper
Grease marking pencils
Labels, adhesive
Lens tissue
Pins, dissecting
Pins, insect
Plastic bags
pH papers or pH meter
Rubber tubing
Varnish, urethane
Vaseline

Apparatus

Aquaria and accessories
Balance
Bunsen burners
Clamps, burette
Forceps, dissecting
Lenses, hand
Microscope (1 per 2 students)
Needles—hypodermic
Needles—innoculation
Needles—dissecting
Probes
Ring supports
Rings
Scalpels
Scalpel blades
Scissors—dissecting
Syringe—disposable
Test tube holders
Test tube racks (40 hole)
Tongs
Trays—dissecting
Wax—for dissecting trays
Wire gauze

Fresh or preserved specimens for laboratory work

collect locally where possible

Algae

Mosses and liverworts

Ferns—sporophytes and gametophytes

Flowering plants as required

Woody stem (tree) sections

Hydra

Tapeworm

Planarians

Earthworms

Grasshoppers

Fish (perch or any local fish)

Frogs—double injected

Microscope slides (prepared)

Spirogyra—conjugating and vegetative

Euglena—w.m.

Amoeba—w.m.

Fern—gametophyte

Roots—c.s. and l.s. (monocot. & dicot.)

Stems—woody and herbaceous, monocot.

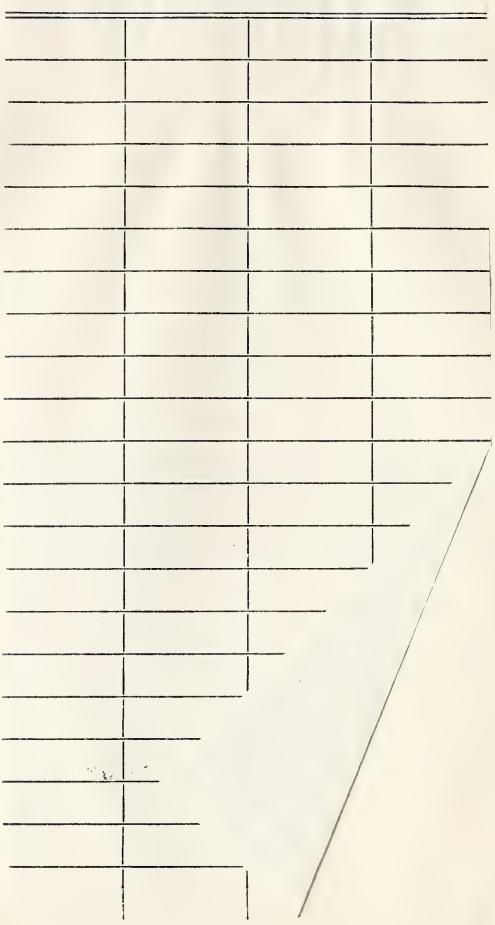
Leaf—angiosperm c.s.

Tapeworm—w.m.

Planarian—w.m. and c.s.

Note: Frequent reference is made to the following book. It is recommended that at least one copy be available for each biology classroom.

Morholt, E., Brandwein, P. J., Joseph, A., *A Sourcebook for the Biological Sciences*. Harcourt, Brace and Company, New York. 1958.



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